Clean Energy Production Technologies

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Bioprocessing for Biofuel Production

Strategies to Improve Process Parameters

Springer

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Series Editors

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The consumption of fossil fuels has been continuously increasing around the globe and simultaneously becoming the primary cause of global warming as well as environmental pollution. Due to limited life span of fossil fuels and limited alternate energy options, energy crises is important concern faced by the world. Amidst these complex environmental and economic scenarios, renewable energy alternates such as biodiesel, hydrogen, wind, solar and bioenergy sources, which can produce energy with zero carbon residue are emerging as excellent clean energy source. For maximizing the efficiency and productivity of clean fuels via green & renewable methods, it's crucial to understand the configuration, sustainability and techno-economic feasibility of these promising energy alternates. The book series presents a comprehensive coverage combining the domains of exploring clean sources of energy and ensuring its production in an economical as well as ecologically feasible fashion. Series involves renowned experts and academicians as volume-editors and authors, from all the regions of the world. Series brings forth latest research, approaches and perspectives on clean energy production from both developed and developing parts of world under one umbrella. It is curated and developed by authoritative institutions and experts to serves global readership on this theme.

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Foreword

Biofuels production is the most sustainable option in renewable energy production pathway for replacing fossil fuels due to its cheap, green, and renewable nature. Although biofuels production from cellulosic biomass is a potentially green and the most effective route, the cost of this technology is still high and far from the practical ground. The high cost of biomass to biofuels production process is mainly contributed by the cost of cellulolytic enzymes and hence needs immediate attention to make this process sustainably viable. It has been inferred from researches till date that in spite of addressing and approaching many issues, this production mode is not sustainably viable and hence we need to focus on "techno-economic analysis" of this process. Techno-economic analysis of the complete biomass to biofuels production process will provide very close visibility about the practical viability of the process, especially the microbial route.

Publication of this book entitled "Bioprocessing for biofuel production: strategies to improve process parameters" is a notable effort in the proposed area. I am happily writing this message with satisfaction as a researcher in the area of biofuels production. This book contains ten chapters addressing the key parameters of biomass to biofuels production technology through microbial route by focusing on their challenges and till date resolving capacity of science. The book presents a consolidated idea about the technical and cost-based gap in this process, which needs to be handled immediately to improve cost economy of biomass to biofuels production process. In my view, this book will prove itself as an asset for the people working and interested in the area, including scientists, researchers, teachers, students, and industrialists.

I appreciate the efforts of Dr. Neha Srivastava (IIT [BHU], Varanasi), Dr. Manish Srivastava (IIT [BHU], Varanasi), Prof. (Dr.) P. K. Mishra (IIT [BHU], Varanasi), and Dr. Vijai Kumar Gupta (TTU, Estonia) for bringing out this book. The efforts taken to complete this book will surely cover the whole and demand of industrialists,

scientists, teachers, researchers, and students. I congratulate the editors for their hard work in bringing this book to its final shape.

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Chapter 1
Impact of Fermentation Types on Enzymes Used for Biofuels Production

Veena Paul, Saloni Rai, Abhishek Dutt Tripathi, Dinesh Chandra Rai, and Aparna Agarwal

1.1 Introduction

Biofuels are a sustainable and renewable source of energy that can be produced from energy crops (like sugarcane and corn), vegetable oil, microbes, organic waste, or biomass. It emits a reduced amount of carbon dioxide as compared to conventional fuels, and in this way, it plays an essential role in lessening the emission of carbon dioxide. Now-a-days, the global energy market has been progressing swiftly because of the reduction of fossil fuels, a perpetual increase in the world population, and industrialized economy. Due to an increase in demand for fuels and its consequent impact of depleting eco-friendly environmental condition and global warming upshots, the development of alternate energy are prime priorities in the research and development area. The bioenergy generated from the biomass signifies a sustainable alternative energy reservoir that gained immense recognition in different divisions from government, public, industries, and researches for its sustainability. The need of these alternative sources is because of toxic gases emission as these gases commence to adverse effects like receding of glaciers, a decline of biodiversity, weather variation, and raise in sea level, and the tremendous requirement for this fossil fuel is additionally affecting the global economic ventures since there is an escalation in the rates of crude oil. The high-speedy modern world progresses by both industrialization and motorization, and it is the primary reason for the inconstant fuel demand. So, promptly the researchers are continuously working in the

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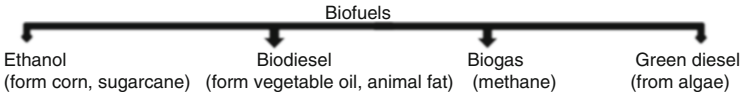
production of sustainable biofuel from sustainable biomass, acknowledging it as an efficient alternative to supersede non-renewable fuels (Gaurav et al. 2017).

1.2 Characteristics of Biofuels

1. It is a type of renewable and carbon-neutral energy source.
2. It releases reduced carbon dioxide apart from the conventional method.
3. It is way of utilization of organic waste or biomass into useful fuel production.
4. It is sustainable due to biodegradable property.
5. It is an efficient energy source.
6. It is non-toxic and environment friendly.

1.3 Classification of Biofuels



Based on origin and production technology, these are classified as follows:

First Generation This generation of biofuels comprises vegetable oils and biodiesel obtained from crop plants. The biofuels of this generation impact adversely on food security; this can overcome by advancing the valuable non-edible feedstock source of biofuels, which then leads to being a cost-effective source for biofuel production.

Second Generation The second generation of biofuels includes bioethanol and bio-hydrogen, and its source of production is agro-waste and non-edible crops.

Third Generation Third generation biofuels involve biobutanol and bioethanol produced from marine reserves, seaweeds, cyanobacteria, and microorganisms.

Fourth Generation This generation of biofuels comprises electro and solar fuels produced by using non-arable land and photosynthetic microorganisms.

1.4 History of Biofuels

The history of biofuels has a lengthy memoir. Firstly, in 1900 a small variant of diesel was produced from peanut oil. In 1920, the implementation of vegetable oil in diesel was started. Then the oil industry has started to employing egg, vegetable oil, and petroleum diesel in diesel. The history of biofuels was started in 1970. Firstly, Austria started the study on biodiesel in 1974 and established a pilot plant producing 500 tons per year of biodiesel using rapeseed oil (Du et al. 2016).

1.5 Biofuel Production Process

In contemporary years, the research is centered on enhancing the yield of biofuel production by using sustainable raw material such as agricultural wastes and biomass. These renewable resources not only enhance the yield of biofuels but also contribute towards the sustainable development of the environment. These sustainable raw materials fulfill high energy demands and minimize the detrimental effect on the environment. The agricultural wastes are used as a renewable raw material and then converted into biofuels in a biorefinery system. The process of bioconversion of these agricultural wastes into valuable products differs because of various parameters like feedstock used and final product. Specific strategies can be implemented in a biorefinery system to prevent hindrance and to enhance production. The biofuel produced are categorized as first-generation and second-generation (Bertrand et al. 2016). Agricultural wastes obtained from cereals, sugarcane, sugar beet, maize, and sorghum are employed for first-generation biofuels production (Obernberger and Biedermann 2012). Agricultural wastes rich in the lignocellulosic matter are employed for producing second-generation biofuels (Kumar and Sani 2018). The bioconversion process for biofuel production involves pre-treatment, hydrolysis, and fermentation (Fig. 1.1).

The pre-treatment of the feedstock is an essential step for biofuel production as it fastens the other steps resulting in higher yield followed by hydrolysis of pretreated substrates and furthers its fermentation for biofuel production (Coyne et al. 2013).

1.5.1 Pre-Treatment

The pre-treatment process is the foremost step in biofuel production. It can be employed by physical, chemical, or biological treatment. The physical pre-treatment involves milling and irradiation; chemical pre-treatment involves acid or alkali treatment, hot-water treatment, steam treatment, microwave, and solvent extraction, and biological pre-treatment involves enzymatic and microbial treatment (Fig. 1.2) (O'Donovan et al. 2013). Lignocellulosic wastes are a rich

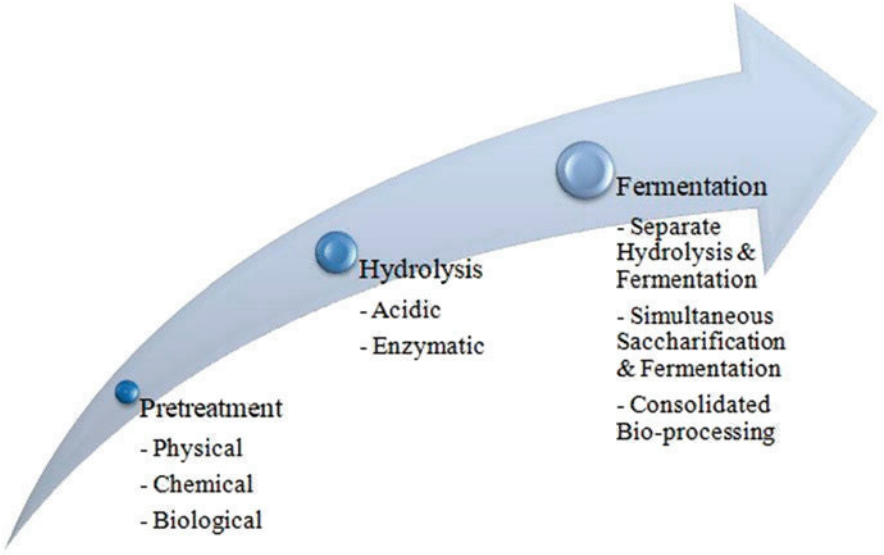


Fig. 1.1 Step involved in biofuel production technology

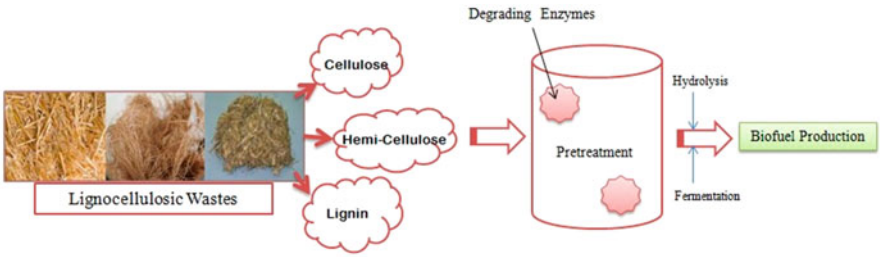


Fig. 1.2 Role of enzymatic pre-treatment for biofuel production

source for biofuel production. For this, the lignocellulosic raw materials are pretreated by steam at high pressure to separate the cellulose, lignin, and hemicelluloses.

Pre-treatment of the raw material can also be done by using chemicals such as organosolv treatment, ammonium fiber explosion (AFEX), and by acid or alkali addition. Generally, sulfuric acid is employed for the pre-treatment to dissolve the hemicelluloses, whereas sodium hydroxide generally used as a source of alkali, which targets lignin. These chemicals also produce various soluble inhibitory compounds due to the degradation of lignin and lead to demerit as it affects the hydrolysis and fermentation process. These inhibitory compounds are toxic, and their toxicity is dependent on the raw material and the conditions of the pre-treatment method (Alvira et al. 2013). These chemical pre-treatment steps also involve various other limitations such as high cost, produce toxic components, pollute the water, and

harm the environment. The microbial pre-treatment is done by implying microorganisms that degrade the lignocellulosic substances (hemicellulose and lignin). These microorganisms produce various enzymes that delignify the lignin component present in the raw material (Coyne et al. 2013). The microbial pre-treatment is advantageous as it reduces the high energy requirements as well as valorizes the waste effectively without any adverse impact on the environment, reduces the release of inhibitors, and is less expensive. However, it also imparts some demerits as it enhances the consumption of cellulose and hemicellulose; hence, time-consuming and can be controlled by the use of various ligninolytic enzymes, which easily hydrolyze lignin. These enzymes also reduce the generation of inhibitory components (Alvira et al. 2013). The method of pre-treatment is selected based on the type of enzymes employed for its hydrolysis. For instance, acid pre-treatment is required as a primary step for the hydrolysis of lignocellulosic raw material from fungal enzymes (Dashtban et al. 2009).

1.5.2 Hydrolysis

In hydrolysis, the pretreated lignocellulosic material is hydrolyzed to yield fermentable sugars like pentoses and hexoses. The biorefinery system comprises two different types of hydrolysis method, viz., acidic and enzymatic hydrolysis. In the acidic hydrolysis method, concentrated or dilute acid is used (like sulfuric acid) to hydrolyze the cellulose. Temperature plays a vital role in this method and depends mainly on the molarity of the raw material (Coyne et al. 2013). Acid generally breaks the hemicellulose and helps in the natural enzymatic breakdown of lignin. Acidic hydrolysis involves two categories, which are diluted acid with high temperature and concentrated acid with low temperature. The latter treatment is more advantageous than the dilute acid process. 30–70% concentrated acid is accounted to yield higher sugar with enhanced biofuel production.

Nevertheless, the concentrated acid treatment leads to dangerous, abrasive, energy-consuming, and costly treatment. The dilute acid treatment has been reported to recover approximately 80–90% of hemicellulose sugars. The acid pre-treatment shows increased sugar release levels when compared to the water pre-treatment method. The demerit of this method is the formation of inhibitors like furans and phenolic compounds, and it also leads to less recovery and adverse environmental effects with concentrated acid and reduced yield with dilute acid. The other method of hydrolysis is an enzymatic method that breaks the lignocelluloses into their respective monomeric sugars. The enzymes produced from bacteria and fungi are used in this method. This method of hydrolysis is a complex process but has no by-product, which is advantageous, but it may exhibit inhibitory effects process of fermentation resulting in fewer yields of biofuels. Hence, this can be controlled by regulating the low pH. In comparison to acid hydrolysis, this hydrolysis method is costly and time taking (O'Donovan et al. 2013).

1.5.3 Fermentation

The hydrolyzed raw material then undergoes fermentation and transforms the hydrolysates (glucose, arabinose, mannose, and xylose) into bioethanol utilizing microorganisms. The microorganisms which are capable of producing ethanol are susceptive to lignocellulosic hydrolysate according to their strain and fermentation provisions (like aeration rate, pH, nutrient requirement, and temperature) (Robak and Balcerek 2018). The inhibitory compounds like phenolics produced during the process of pre-treatment and hydrolysis are detoxified before the fermentation step. Saccharomyces cerevisiae is primarily used in biorefinery processing due to its efficient recombinant techniques and high fermentation rate (Coyne et al. 2013). For example, TMB 3400 efficiently converts glucose, xylose, and arabinose into an enhanced yield of bioethanol (Dashtban et al. 2009). Further, to achieve higher fermentation yield, the biorefinery processing steps, viz., pre-treatment, hydrolysis, and fermentation are combined to get effective enhanced yield with low cost and less time-consuming. The combination method can be categorized as follows:

- Separate Hydrolysis and Fermentation (SHF)—Optimizes each process separately but uses a large number of enzymes implicated in biofuel production, which thus make this process costly.
- Simultaneous Saccharification and Fermentation (SSF)—This method results in direct fermentation of hydrolysates into biofuel by combining the saccharification and fermentation process into one reaction. In this process, both the hydrolysis and fermentation step undergo concurrently.
- Consolidated Bioprocessing (CBP)—This method involves all the three steps of cellulase production, hydrolysis, and fermentation together by utilizing one or more than one cellulolytic microorganisms. This method is less expensive than other methods and only requires optimized pH, temperature, enzymes, and microorganisms.

1.6 Enzymes in Biofuel Production

The best and cheaper source of biofuel production is lignocellulosic substrates. These lignocellulosic-rich raw materials are complex, and it is difficult to degrade these compounds. So, it is necessary to alter the complex polymers into a more straightforward form, which is a challenging task in the biofuel production industry. Several physical, chemical, and biological pre-treatments are employed for the conversion of the complex polymer. Enzymatic treatment is one of the best methods and a green approach toward the eco-friendly and sustainable production of biofuels and it provides high specificity and requires less energy. Enzymes like cellulase convert the cellulose and xylanases convert the hemicellulose into sugar, which is further fermented by the various groups of microorganisms for biofuel production. The different enzymes used for biofuel production are listed in Table 1.1.

Table 1.1 List of enzymes associated in biofuel production

SN	Lignocellulosic biomass	Group of lignocellulosic degrading enzymes	Enzymes involved in degradation	E.C. Number
1.	Cellulose	Cellulases	Endo-glucanases	EC 3.2.1.4
			Endo-1, 4-β-xylanase	EC 3.2.1.8
			β-Glucosidases	EC 3.2.1.21
			β-Xylosidase	EC 3.2.1.37
			α-Arabinofuranosidases	EC 3.2.1.55
			Cellobiohydrolase	EC 3.2.1.91
2.	Hemicellulose	Hemicellulases	Mannanases	EC 3.2.1.78
3.	Lignin	Ligninases	Manganese peroxidase	EC 1.11.1.13
			Lignin peroxidase	EC 1.11.1.14
			Versatile peroxidases	EC 1.11.1.16
			Catechol oxidases	EC 1.10.3.1
			Laccase	EC 1.10.3.2
			Glyoxal oxidase	EC 1.2.3.5
			Aryl alcohol oxidase	EC 1.1.3.7

The complex lignocellulosic substrates are unable to be degrading by a single enzyme and thus require a series of enzymes for its complete hydrolysis. The enzyme degrades the lignocellulosic substrates and allows the easy availability of cellulose, hemicellulose, and lignin to the fermenting microorganism for biofuel production (Fig. 1.3).

The vital enzymes employed in the hydrolysis of lignocellulosic substrates are categorized as cellulases, hemicellulases, and ligninase. These enzymes cleave the bonds and thus degrade the cellulose, hemicellulose, and lignin.

Cellulases (EC 3.2.1.4) This enzyme plays an essential role in the degradation of the cellulosic component. This enzyme is comprised of endo-glucanases, cellobiohydrolase, and β-glucosidases. These enzymes are extracellular enzymes isolated from the group of fungi. Some of the cellulose producing microorganisms can produce cellulosomes (an extracellular multi enzymatic complex) that can degrade cellulose and hemicelluloses. The cellulase enzymes are categorized in

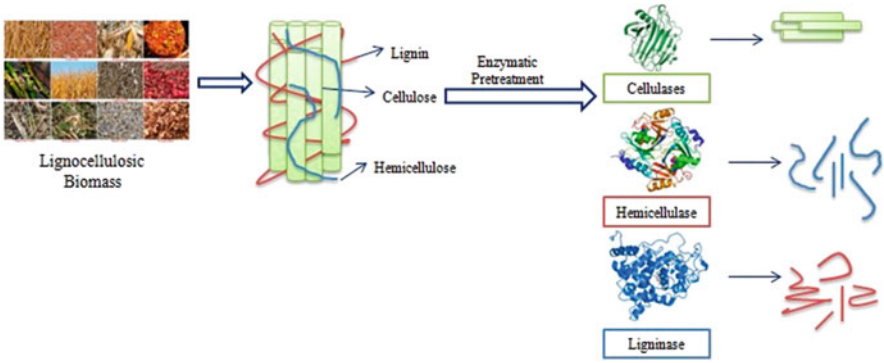


Fig. 1.3 Mechanism of enzymes in degradation of lignocellulosic biomass

11 glycoside hydrolase families and are composed of the catalytic and carbohydrate-binding module that can hydrolyze cellulose polymer into glucose monomers and are mainly produced from a fungal source. For the complete degradation of cellulose into glucose, all three cellulolytic enzymes show a synergistic effect.

- *Endo-glucanases (EC 3.2.1.4)*—Degrade cellulose by breaking the β-1, 4 linkages within the chain at amorphous sites, and liberate oligosaccharides. These enzymes are monomeric proteins that cleave the β-1, 4-glycosidic bonds of the cellulose chains.
- *Cellobiohydrolases (EC 3.2.1.91)*—These exo-acting enzymes are monomeric. They split cellobiose from their non-reducing and reducing chains. These mainly cleave the long-chain oligosaccharides produced by the action of endo-glucanases enzymes.
- *β-glucosidases (EC 3.2.1.21)*—Degrade smaller chains of oligosaccharides by unleashing the β-D-glucosyl residue. These cellulolytic enzymes are capable of hydrolyzing cellobiose yielding glucose. These enzymes can be categorized as extracellular, intracellular, and cell wall associated groups with molecular masses of 35 kDa (monomeric protein) or more than 146 kDa (di- or trimeric protein).

The enzymatic hydrolysis of cellulose from lignocellulosic substrate takes place in two steps, viz., primary and secondary. The primary hydrolysis step comprises two enzymes, namely, endo-glucanases and cellobiohydrolases. These enzymes require a degree of polymerization up to 6 for the release of sugars. Both enzymes act together in a cellulose-binding and catalytic domain. The secondary hydrolysis involves β-glucosidase for the production of glucose from cellobiose.

Xylanases (EC 3.2.1.8) Xylanases hydrolyze lignocellulosic materials. These enzymes break xylan heteropolymers from xylooligosaccharides into xylose with the help of accessory enzymes like β-xylosidases and endo-1, 4-β-xylanases. The xylan chains consist of β-1, 4-glucosidic bonds, which is hydrolyzed by endo-1, 4-β-xylanases, whereas β-xylosidases hydrolyze the xylobiose and xylooligomers. This xylanases enzyme hydrolyzes the xylan, which is an essential component of

hemicellulose. The use of this enzyme leads to an eco-friendly approach to the degradation of xylan. Xylanase enzyme is mainly produced from *Bacillus* sp., *Trichoderma reesei,* and *Humicola insolens* at an optimum temperature of 40–60 °C. The xylanase enzyme consists of a sequence of the enzyme having a synergistic effect for the conversion of xylan into sugars and involves endo-1, 4-β-xylanase, β-xylosidase, esterases, and α-arabinofuranosidases (Binod et al. 2019).

- *Endo-1, 4-β-xylanase (EC 3.2.1.8)*—is one of the critical enzymes for the degradation of xylan.
- *β-xylosidase (EC 3.2.1.37)*
- *α-arabinofuranosidases (EC 3.2.1.55)*—these enzymes help in the removal of arabinose and 4-O-methyl glucuronic acid from xylan.

Esterases These enzymes cleave the ester linkage between acetic acid and xylose present in xylan (Acetoxylan esterase EC 3.1.1.72). This enzyme also eliminates O-acetyl from acetyl xylan, which is rich in β-D-pyranosyl residues. Other esterases enzymes involved are ferulic acid esterase (EC 3.1.1.73), this mainly cleave the ferulic acid side chains and mainly act between arabinose and p-coumaric acid (Binod et al. 2019).

Ligninases Lignin is the crucial component of lignocellulosic biomass. For the bioconversion of these substrates into biofuels, it is essential to degrade the complex polymers of lignin. These groups of enzymes comprise lignin peroxidase, manganese peroxidase, laccase, and versatile peroxidase.

Lignin Peroxidase (EC 1.11.1.14) This enzyme lignifies, degrades, and depolymerizes the lignin content synergistically. Lignin peroxidases are heme-rich hydrogen peroxide-dependent enzyme accountable for the oxidation of the lignin component of high redox potential. These enzymes are capable of oxidizing non-phenolic lignin compounds. This enzyme non-specific and extracellular, obtained from white-rot fungi (*Phanerochaete chrysosporium*). The molecular mass of lignin peroxidase is 40 kDa, which forms a monomeric protein (Niladevi 2009). This enzyme comprises iron combined with four tetrapyrrole rings and residues of histidine. Lignin peroxidase oxidizes multiple phenolic compounds (vanillyl alcohol, guaiacol, and syringic acid) and non-phenolic compounds. This enzyme also contains tryptophan residues on the surface of trp171 enzyme, which contributes to the transfer of electrons from aromatic substrates leading to oxidation of lignin by cleaving of non-catalytic bonds. The critical element of the lignin peroxidase enzyme is hydrogen peroxide, which helps to degrade the lignin.

Manganese Peroxidase (EC 1.11.1.13) This category of the enzyme is classified as hydrogen peroxide-dependent heme-containing peroxidase enzymes able to degrade lignin compounds. The enzyme was first isolated from *Phanerochaete chrysosporium* with 40–50 kDa molecular mass. Manganese acts as a cofactor for this enzyme for the sufficient oxidation of lignin compounds.

Versatile Peroxidases (EC 1.11.1.16) This enzyme is isolated from *Pleurotus* sp. with the ability to oxidize phenolics and non-phenolic aromatic compounds and manganese. These enzymes are similar to manganese peroxidases in terms of their structure and the binding site for manganese. This enzyme also possesses residues of tryptophan, which is essential for the electron transfer from aromatic lignin substrates.

Laccase (EC 1.10.3.2) The oxidoreductase enzyme laccases are extracellular glycoproteins of superfamily multi-copper oxidase (MCO). MCO is known to produce laccases from different sources like plants, microorganisms, and some insects. These enzymes are obtained from white-rot fungi and ascomycetes (Lundell et al. 2010). This MCO catalyzes the oxidation reaction of phenolic and non-phenolic lignin with the collateral conversion of molecular oxygen to water. Martínez et al. (2005) reported that laccases enzyme could also be obtained from brown-rot fungi. A report from Piontek et al. (2002) states that the basidiomycetes *Trametes versicolor* are responsible for the molecular structure of laccases. The molecular mass of fungal laccases ranges from 60–80 kDa having 3–6 pI (isoelectric point). Laccase enzyme includes three regions, namely, D1, D2, and D3, typically bounded with copper atoms. Depending on the number of copper ions existing on the active site of laccases, it gives white, yellow, and blue color (De Blasio 2019). Blue laccases are referred to as true laccases because of the ubiquity of all four copper ions. White laccases generally comprise one copper ion, while yellow laccases do not hold a Type I copper atom. These laccases are referred to as non-true laccases and contain metal ions like zinc, iron, and manganese instead of copper ions. For example, POXA1, obtained from *Pleurotus ostreatus*, produces white laccases that comprise one copper ion, one iron ion, and two zinc ions (Baldrian 2006). The laccase glycoproteins have reported secreting numerous isozymes superimposed multiple gene encoding. White-rot fungi *Ganoderma lucidum* secretes five different isozymes (D'Souza et al. 1999). Laccases play an essential role in degrading lignin by catalyzing the redox reaction of the phenolics. Fungal laccases are broadly used in the bioprocessing of fuels and function as lignin biodegradation and depolymerization through the oxidation of phenolics components. Laccases have low redox potential but act as a natural biocatalyst because of its molecular oxygen. At the active site of the laccase enzyme, the T1 copper ion is related to the substrate oxidation and collateral reduction of the copper ion, accompanied by the transfer of the electron to the T2 and T3 trinuclear cluster of copper ion, this leads to the substrate oxidation with the free radical production. Then the free-electron coalesces amid the molecular oxygen to form a water molecule.

Hydrogen Peroxide Producing Enzymes During the process of lignin degradation, extracellular peroxidase enzymes need hydrogen peroxide for active degradation. The hydrogen peroxide is formed due to the lessening of molecular oxygen into hydrogen peroxide.

Glyoxal Oxidase (EC 1.2.3.5) This oxidase enzyme possesses copper ion and oxidizes various co-substrates (aldehydes), for instance, methylglyoxal and glyoxal.

Aryl Alcohol Oxidase (EC 1.1.3.7) This flavoenzyme is isolated from *P. eryngii*, help to ascend the content of hydrogen peroxide. For example, this enzyme oxidizes chlorinated anisyl alcohols throughout the process of lignin degradation.

Phenol Oxidases This enzyme is categorized under copper-containing enzymes that conceal the activity of peptidase and glycosyl hydrolase. In the presence of molecular oxygen, phenol oxidase oxidizes various phenolic compounds.

Tyrosinosis These enzymes are homo-tetrameric proteins having four copper ions. This enzyme is having catalytic property and shows cresolase activity and catechol oxidase activity by catalyzing the o-hydroxylation of monophenols to o-diphenol, followed by o-quinone. The molecular mass of this enzyme is 60 kDa. This enzyme is suitable for substrate rich in tyrosine, catechol, and L-DOPA (L-3,4-dihydroxyphenylalanine).

Catechol Oxidases (EC 1.10.3.1) This enzyme is similar to tyrosinosis, having a molecular mass of 60 kDa but do not possess cresolase activity. The enzyme catechol oxidase is a crucial factor in melanin synthesis. This enzyme is formed by two copper ions attached to three histidine residues. This enzyme principly catalyzes the oxidation of o-diphenols to o-quinones. The substrate rich in catechol, chlorogenic acid, catechin, and caffeic acid is of interest for this enzyme.

Catalase-Phenol Oxidases These are bifunctional antioxidant enzymes isolated from the ascomycetes class of fungi having tetrameric heme-containing proteins with 320 kDA molecular mass. These enzymes show catalase activity (able to decompose hydrogen peroxide) and are capable of oxidizing o-diphenolic compounds (in the lack of hydrogen peroxide). These enzymes are useful for the substrates rich in L-DOPA, catechol, chlorogenic acid, catechin, and caffeic acid.

Hemicellulases These are the enzymes having the ability to degrade hemicellulose. The pre-treatment of hemicellulose by acid or hydrothermal method (like a steam explosion) results in composition and structural modifications while the alkali pre-treatment (like ammonia fiber/freeze explosion) and biological methods are found to be less effective.

Mannanases (EC 3.2.1.78) This enzyme degrades mannan-rich hemicelluloses and is constituted of β-1, 4-mannanase, and β-mannosidases, which break the glucomannan/galactomannan and mannan substitutes.

Proteins in Biofuel Production Swollenins, these enzymes break the crystalline structure of cellulose but do not hydrolyze cellulose and hemicellulose. These are similar to expansins and degrade the cellulose by breaking the hydrogen bonds. Expansins are plant-derived proteins that control the prolongation of the plant cell wall and help to degrade the lignocellulosic compounds and utilize cellulases for increased hydrolysis of cellulose.

1.7 Kinetics of Biofuel Synthesis

Kinetic modeling is a notion for the modeling of various reactions involved in biofuel production. It is an important parameter to study the reaction kinetics of enzymes implicated in the biofuel production as well as to study each elementary reaction involved in the process. Due to the complicated processing steps involved in biofuel production, the kinetic model is a solution to enhance production. The kinetic modeling involves a different kind of reactions and kinetic constants. The kinetic modeling can be employed in combination with various computational software tools, which decreases the complexity of the process (Vasquez and Eldredge 2011). These kinetic models also play a vital role in the mathematical evaluation of the fermentative processes leading towards large-scale simulation. The kinetic model controls various factors involved in the fermentation process (like specific growth rate and biomass yield) is vital for biofuel production. The kinetic modeling for biofuel synthesis is crucial as it helps to determine various essential factors like specific growth rate, biomass yield, productivity, process control, and scale-up (Rodríguez-León et al. 2018).

Generally in kinetic modeling, the kinetic constant of the reaction involved in biofuel processing is evaluated (Gagliano et al. 2018). The kinetic constant required for evaluating the chemical reaction involved in the bioconversion process at a constant rate can be computed by Arrhenius mathematical expression for kinetic constant:

$$k(T) = A_{\exp}\left(-\frac{E_a}{RT}\right)$$

where $k(T)$ is kinetic constant, $A_{\exp}$ is the pre-exponential factor, E_a refers to activation energy, R is the ideal gas constant, and T is the absolute temperature.

One of the critical parameters during the fermentation process is the specific growth and its evaluation. For the kinetic study of the specific growth, it is first considered as a variable that will be linked to other dependent or independent factors. The identification of various factors involved will lead to symbolize the kinetic process. For instance, during the fermentation process for biofuel production, the variable must set up a dynamic relationship with the variation by other factors involved in the fermentation process (like biofuel synthesis, substrate concentration, amount of oxygen consumed, and the final product).

A specific variation to determine the kinetics of a process is dependent on different factors given as equation:

$$\frac{dX}{dt} = f(S; T; \text{etc.})$$

where X is the concentration of biomass (gram per liter); t is the time (hours); S is the substrate (gram per liter), and T is the temperature (degree Celsius).