# ENZYMATIC PRETREATMENT OF LIGNOCELLULOSIC BIOMASS FOR BIOGAS PRODUCTION - REVIEW

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**Abstract.** Currently, due to global concerns regarding energy security and its environmental consequences, biomass has been promoted as an important resource for reducing greenhouse gas emissions through its conversion into biofuels. The main limitation in the use of lignocellulosic substrate for biofuel production is its resistant structure. Pretreatment is a necessary step to overcome the lignocellulosic biomass rigid structure and to improve its biodegradability for energetic conversion (biomethane, biohydrogen, bioethanol, etc.). Lately, a number of pretreatment methods have been studied and applied, such as physical, thermal, chemical and biological pretreatment which can be used individually or in combination. Compared to physical and chemical pretreatment techniques, biological pretreatment usually requires much lower energy consumption and does not involve the use of chemicals, which presents economic advantages and a minimal impact on the environment. The anaerobic digestion process has proven to be an effective technology for converting organic substrate into energy in the form of biogas. Including a pretreatment technique in anaerobic digestion processes increases lignocellulosic biomass digestibility and improves the biogas yield. The aim of this review is to give an overview on the enzymatic pretreatment applied to lignocellulosic biomass substrate used for biogas production. There are presented different types of enzymes, namely cellulase, lipase, amylase, protease, and ligninolytic enzymes which are responsible for the degradation of lignocellulosic substrate. The article includes information related to the structure of lignocellulosic biomass, the advantages of biological pretreatment, as well as the current state of research in the direction of enzymatic treatment to enhance the anaerobic digestion process.

**Keywords:** lignocellulosic biomass, biological pretreatment, enzymes, anaerobic digestion.

## Introduction

The global decrease in fossil fuel resources, as well as their negative impact on the environment, have created premises for the identification and development of new, sustainable, economic and non-polluting sources of energy [1; 2].

The unsustainable consumption of global resources represents a serious threat to the environment, i.e climate change, global warming, and also creates a negative impact on the human health. In line with that, the European Union supports the development and adoption of policies that promote the use of renewable energy resources [3]. In this regard, the main focus is to minimize air pollution caused by greenhouse gases, especially carbon dioxide ($CO_2$).

Considering several alternative energy sources, biomass has proven to be a promising substrate for conversion into biofuels for transportation, electricity, heat and into bio-based products [1; 4]. Currently, biomass represents the most abundant form of renewable energy and includes all organic matter, such as agricultural and forestry residues (waste from the wood processing industry: shavings, sawdust), animal residues (from animal farms), sewage sludge, algae, aquatic cultures and organic fraction of municipal solid waste. From an energetic point of view, the term “biomass” refers to organic matter that can be converted into energy. It is important to note that $CO_2$ generated during biomass combustion does not contribute to an increase in atmospheric $CO_2$, making it a fully renewable energy source [4; 5].

The energy production from biomass involves different technologies for organic waste valorisation (gasification, anaerobic digestion, and transesterification are some of the methods). Among them, anaerobic digestion and fermentation are two of the most common methods for converting biomass into useful products. The anaerobic digestion process is considered to be a key technology for the sustainable use of biomass. Anaerobic digestion is a biological process in which organic substrate is decomposed in the presence of several bacteria species, under controlled environmental conditions, in the absence of oxygen. The biogas production through the anaerobic fermentation process is currently the most widespread practice in Europe. Biogas is a renewable and ecological fuel with wide applications worldwide and tending to reduce greenhouse gas emissions and mitigate climate change [2; 6-8].

The main stages of the anaerobic digestion process of lignocellulosic biomass for biogas production are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The process efficiency is significantly influenced by a number of factors, such as the temperature, pH value, carbon/nitrogen ratio, substrate biodegradability, organic loading rate, hydraulic retention time, substrate mixing and inhibitors. It has been demonstrated that the anaerobic digestion process is less effective when the used substrate has a high cellulose concentration [9; 10].

The processes used in the energy conversion of biomass can be divided into four main categories, namely: thermo-chemical (combustion, pyrolysis, gasification, and liquefaction) [11; 12], biochemical (anaerobic digestion and fermentation) [7; 13], chemical (transesterification) [14] and physical methods (biomass pelleting and briquetting) [15; 16].

The main disadvantage of converting lignocellulosic biomass into simple sugars is the fact that lignin is resistant to breakdown. Thus, pretreatment is an indispensable step in the conversion of lignocellulosic biomass into fermentable sugars for biofuel production [17].

The aim of this review is to give an overview on the enzymatic pretreatment applied to lignocellulosic biomass substrate used for biogas production. There are presented different types of enzymes, namely cellulase, lipase, amylase, protease, and ligninolytic enzymes which are responsible for the degradation of lignocellulosic substrate. The article includes information related to the structure of lignocellulosic biomass, the advantages of biological pretreatment, as well as the current state of research in the direction of enzymatic treatment to enhance the anaerobic digestion process.

## Pretreatment methods applied to lignocellulosic biomass

Lignocellulosic biomass has a great potential to produce biofuels as well as value-added bioproducts in the context of circular economy and bioeconomy [3]. The three primary components of lignocellulosic biomass are cellulose (30–60% of dry matter), hemicellulose (14-40% of dry matter) and lignin (7–25% of dry matter). Lignin and cellulose content is higher in woody biomass, while lignocellulosic grass-type biomass has a higher proportion of hemicellulose. The percentage of the three fractions mentioned above varies according to the type of biomass [18]. Besides the three primary components of lignocellulosic biomass, there are other components such as pectin, lipids, proteins, starch, minerals, water, inorganic substances, as well as extractives (tannins, lipids, resins, steroids, terpenes, terpenoids, flavonoids, and phenolic compounds). Biomass, in its natural form, is difficult for use in energy production, therefore pretreatment is necessary to overcome its recalcitrant nature and make it suitable for conversion. Pretreatment leads to physical, chemical and structural changes in the biomass and plays a fundamental role in the viability of the biomass energy production process. Moreover, biomass pretreatment is important to break the lignin structure and the crystalline structure of cellulose, in order to transform it into a substrate accessible to enzymes [3; 19].

Various pretreatment methods are now available to solubilize, hydrolyze and separate the cellulose, hemicellulose and lignin components. These include physical (mechanical), chemical, physico-chemical and biological pretreatments [20]. Some researchers have reported that biological pretreatment of lignocellulosic biomass represents one of the most attractive pretreatments since it is environmentally friendly, has low operating costs, and mild operating conditions [21; 22].

Compared to physical and chemical pretreatment methods, biological pretreatment usually requires much lower energy consumption and does not involve the use of chemicals, which presents economic advantages and a minimal impact on the environment [23]. In addition, regarding the cost of the pretreatment methods, the authors Kamusoko et al. [24], in their study concluded that both biological pretreatment and combined pretreatments are cost-effective, the rest of the methods being classified as expensive.

## Enzymatic pretreatment of lignocellulosic biomass for enhancing biogas yield

The main purpose of the lignocellulosic biomass biological pretreatment is to find the microbial species that de-lignify efficiently the used substrate and that are advantageous for the anaerobic digestion process. Besides enzymatic pretreatment applied to lignocellulosic biomass, there are another two methods that are used as biological pretreatment, namely bacterial and fungal pretreatment. The main benefits of biological pretreatment include low energy consumption, simpler technology, and inexpensive operation costs [25]. Enzymatic pretreatment of lignocellulosic biomass into its monomeric components is considered the most sustainable method. The influence of enzymes on the treated lignocellulosic biomass depends on the type of enzymes used, also on the biomass composition. There are several enzymes tested in the biological pretreatment, such as ligninolytic enzymes (laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP)), cellulase, lipase, amylase, and protease [22; 26].

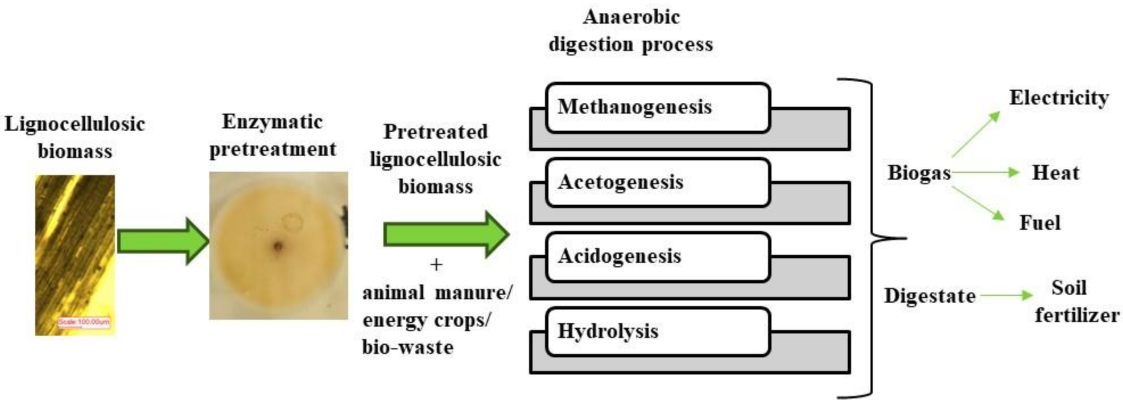


Fig. 1. **Enzymatic pretreatment of lignocellulosic biomass for improving biogas production**

*Cellulase* is a class of enzymes made up of endoglucanase and exoglucanase including cellobiohydrolases as well as β-glucosidase. A variety of microorganisms, such as actinomycetes, bacteria, and fungi, secrete cellulases. The most important producers of cellulase include species such as *Trichoderma*, *Aspergillus*, *T. viride*, *A. niger*, and *Penicillium*. The decomposition of cellulose polysaccharide is catalysed by cellulase, with simple breakdown of β-1,4-glycosidic linkages [22; 27]. Studies showed that cellulase obtained from *Trichoderma reesei* is known for its capacity to degrade insoluble cellulose, which is present in lignocellulosic biomass [27; 28].

Another enzyme used, *laccase*, has been shown to play an important role in lignin degradation, being widely found in nature, produced particularly by fungi and bacteria and being the most investigated enzymatic system. Laccase oxidizes lignin which breaks it down into smaller molecules so than other enzymes can more easily degrade. It has also been proven that laccase can break down other biomass components, such as cellulose and hemicellulose [29; 30]. Laccases are a remarkable class of multicopper enzymes that oxidize both phenolic and non-phenolic lignin-related compounds as well as highly recalcitrant aromatic compounds [31].

Lately, there has been increasing interest in using enzymatic pretreatment to degrade lignocellulosic biomass components. The effect of enzymatic pretreatment of sugar beet pulp and spent hops prior to methane fermentation was determined in their research by Ziemiński et al. [32]. The authors reported that the biogas production was increased by the enzymatic treatment by 19% and 13%, respectively (versus relevant controls). The highest biogas yield was generated by sugar beet pulp. Curran et al [33] assessed the different innovations of lignin valorization using laccases within the context of a biorefinery process. In other study, Deng et al. [34] investigated the effects of laccase pretreatments on physicochemical characteristics of alkali lignin and wheat straw. Their results showed that the reducing sugar yield during subsequent enzymatic hydrolysis increased by 26% after laccase-mediated system treatment.

LiP and MnP are other types of ligninolytic enzymes; MnP and laccase can only oxidize phenolic compounds, while LiP can catalyze both phenolic and non-phenolic units. LiP and MnP have been defined as true lignin degraders due to their high redox potential. Ligninolytic enzymes are produced by various fungi, such as brown-rot, white-rot, and soft-rot fungi, white-rot fungi being the most active during biological pretreatment [26].

*Lipase* is another enzyme that is utilized to improve the generation of biogas. Lipases are very versatile enzymes and can be obtained from a variety of sources, such as microbiological, animal, and vegetable. *Pseudomonos*, *Bacillus*, *Arthrobacter* and *Alcaligenes* species of bacteria and *Aspergillus*, *Fusarium*, and *Penicillium* species of fungi are extensively tested for the production of lipase. Lipases have the property to catalyze the hydrolysis of long chain triglycerides. As a versatile biological catalyst, lipases have provided a positive perspective for fulfilling the requirements of different industries, such as biofuel production, textile, pharmaceuticals, foods and drinks [22; 35]. In their research, Kameswari K.S.B. et al. [36] used steapsin, a commercial grade lipase, to enhance the hydrolysis in anaerobic codigestion of fleshings and a mixture of primary and secondary sludge generated during the treatment of tannery wastewater. The results showed that the optimal dose of lipase was about 0.75 g. The biogas generation increased by about 15% compared to the process without adding lipase.

The primary enzyme responsible for catalyzing hydrolysis of amylopectin and amylose is *amylase*. The amylase enzyme can break down carbohydrates by breaking bonds between sugar molecules in polysaccharides through a hydrolysis reaction. Nugraha et al. [37] analyzed the effect of pretreatment using amylase and cellulase enzymes on the production of biogas from rice husk substrate. Their results showed that pretreatment with the addition of amylase and cellulase enzymes increased biogas production. The highest biogas production was obtained by pretreatment of 18% amylase enzyme, 1466 mL, and by pretreatment of 18% cellulase enzyme, 1075 mL. In another study, Wang et al. [38] reported that the methane yield of starch increased by 16.0% through amylase pre-treatment. Dima and Mateescu reported that the enzymatic addition of 1% (w/w) α-amylase and 1% proteinase K to the sunflower seed cake substrate increased biomethane with about 8.5% compared to the control test [39].

*Proteases* are the extracellular enzymes that are capable to hydrolyze proteins into their constituent amino acids and polypeptides. Bonilla et al. [40] investigated the potential for enzymatic pretreatment on the anaerobic digestibility of pulp mill biosludge. They observed a maximum improvement of 26% in the biogas yield after 62 days of biosludge digestion when pretreated with protease from *B. licheniformis*.

## Conclusions

1. The anaerobic digestion process represents one of the most promising options for the management and valorization of lignocellulosic waste, thus supporting the circular economy.
2. Biomass can be converted into liquid, solid and gaseous biofuel using innovative methods, thus becoming a clean energy as a clean and effective energy source for a variety of applications, including transportation fuel, heating and power generation.
3. The main aim of all lignocellulosic biomass pretreatment methods is to fully breakdown lignin, cellulose and hemicellulose into smaller particles that are easily accessible to enzymatic hydrolysis.
4. Biological pretreatment could represent an efficient solution for lignocellulosic biomass pretreatment, due to its low cost, low energy requirements, eco-friendly nature, mild environmental conditions, and the absence of inhibitor generation during the anaerobic digestion process; the use of enzyme during pretreatment has the ability to solve the operational problems of anaerobic digestion process.

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## Author contributions:

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