

PRETREATMENTS AND FACTORS AFFECTING SACCHARIFICATION AND FERMENTATION FOR LIGNOCELLULOSIC ETHANOL PRODUCTION

LIN MYAT and GI-HYUNG RYU

*Department of Food Science and Technology, Kongju National University, Yesan,
Choongnam 340-802, Korea*

✉ *Corresponding author: Gi-HyungRyu, ghryu@kongju.ac.kr*

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Lignocellulosic biomass can be utilized to produce ethanol, a promising alternative energy source for sustainable energy production and has the potential to be a valuable substitute. Various pretreatment techniques change the physical and chemical structures of the lignocellulosic biomass and improve the hydrolysis rate. The cost of ethanol production from lignocellulosic material is relatively high based on current pretreatment technologies and the main challenges are low yield and high cost of the hydrolysis process. Considerable research efforts have been made to improve the hydrolysis of lignocellulosic biomass. There are many factors involved in the enzymatic hydrolysis (saccharification) and fermentation of lignocellulosic biomass. In this review, different kinds of pretreatment and factors affecting enzymatic saccharification and fermentation in the industrial ethanol production are highlighted for the development of ethanol production in the future. Thermomechanical extrusion, particle-size reduction, and alkaline pretreatments are potential pretreatments for lignocellulosic ethanol production. Crystallinity index, lignin and hemicellulose contents, and degree of polymerization are the main factors affecting enzymatic saccharification. Total phenolic content and furfural mainly affect fermentation. Protein digestibility and free amino nitrogen are the essential nutrients for yeast during fermentation.

Keywords: pretreatments, enzymatic hydrolysis, fermentation inhibitors, essential nutrients

INTRODUCTION

Efficient conversion of lignocellulosic materials into ethanol has become a world priority for producing environmentally and friendly renewable energy. Lignocellulosic materials are composed of carbohydrate polymers (cellulose, hemicellulose), lignin and extractives and minerals that are recalcitrant to deconstruction. Two thirds of lignocellulosic materials are made up of cellulose and hemicellulose, which are the substrate for second generation ethanol production. In their native conformation, cellulose and hemicelluloses are largely protected from enzymatic degradation due to associations of these polymers with lignin and with each other, acting as a barrier and interfering with hydrolysis.¹

The pretreatment is a crucial process step for the conversion of lignocellulosic biomass into bioethanol. It is required to alter the structural and chemical composition of lignocellulosic biomass to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars.²

The modification of a lignocellulosic substrate is achieved through various pretreatment technologies that disrupt the cell wall structure and make it accessible to enzymes. The aim of the pretreatment is to break down the lignin structure and the crystalline structure of cellulose, so that the enzyme can easily access and hydrolyze the cellulose.³ The pretreatment is an important step in lignocellulosic ethanol production, and efficient and economical pretreatment technologies for lignocellulosic ethanol production will play a major role in conversion of biomass to ethanol.

Moreover, a critical step in lignocellulosic biomass conversion to ethanol is the hydrolysis of cellulose and hemicellulose to fermentable sugars, which is affected by numerous factors, including composition and structure of the feedstock, pretreatment method, type and loading of enzyme, cellulose crystallinity, and available surface area.⁴ Initial cellulose materials exhibit a high resistance to enzymatic hydrolysis. Various structural

factors that limit the ability of cellulose to hydrolysis are cellulose crystallinity, accessible surface area, protection by lignin, hemicellulose content and degree of polymerization.

Depending on the process and conditions using different pretreatments, hemicellulose sugars may be degraded to weak acids and furan derivatives, which potentially act as microbial inhibitors during fermentation. Lignin derived products can also be formed and further interfere along the process.⁵ The basis of inhibition appears to be a lack of sufficient protein synthesis and nitrogen in the mash required to support an accelerated fermentative metabolism in *Saccharomyces*. Therefore, in this paper a review on different pretreatment technologies and factors affecting enzymatic saccharification and fermentation for lignocellulosic ethanol production is presented.

PRETREATMENT TECHNOLOGIES FOR LIGNOCELLULOSIC BIOMASS

The digestibility of cellulose present in lignocellulosic biomass is hindered by many physicochemical, structural, and compositional factors. In the conversion of lignocellulosic biomass to ethanol, the biomass needs to be treated so that the cellulose in the biomass is exposed. Several methods have been developed for pretreatment of lignocellulosic materials prior to enzymatic hydrolysis. These methods are classified into biological, physical, chemical, and a combination of these methods.

Biological pretreatments

Biological pretreatment employs microorganisms, mainly brown, white and soft-rot fungi, which degrade lignin and hemicellulose and very little of cellulose, more resistant than other components.⁶ Biological pretreatment can be used not only for lignin solubilisation, but also for the removal of specific components, such as antimicrobial substances. Lignin degradation by white-rot fungi, the most effective for biological pretreatment of lignocellulosic materials, occurs through the action of lignin-degrading enzymes, such as peroxidases and laccases.⁷ Biological pretreatment by white-rot fungi has been combined with organosolv pretreatment in ethanol production by simultaneous saccharification and fermentation from beech wood chips.⁸ Results from other recent studies have shown that fungal pretreatment of wheat straw for 10 days with a high lignin-degrading and low cellulose-degrading fungus results in a reduction in acid

loading for hydrolysis, and an increase in the release of fermentable sugars and a reduction in the concentration of fermentation inhibitors.

Low energy consumption, no chemical requirement and mild environmental conditions are the main advantages of biological pretreatment. However, the main drawback to develop biological pretreatment is the low hydrolysis rate obtained in most biological materials compared to other pretreatment technologies.

Physical pretreatments

Mechanical particle-size reduction

The objective of mechanical pretreatment is a reduction of particle size and crystallinity of lignocellulose in order to increase the specific surface area and reduce the degree of polymerization. This can be produced by a combination of chipping, grinding or milling depending on the final particle size of the material.⁹

The power requirement of this pretreatment is relatively high depending on the final particle size and the biomass characteristics. Taking into account the high energy requirement of milling and the continuous rise of energy prices, this technology is not economically feasible.¹⁰ Particle-size reduction by grinding is widely used as a first step in increasing the specific surface area of the substrate and making it more amenable to enzymatic attack. However, particle-size reduction alone is not sufficient to modify the lignocellulosic structure and it is often necessary to combine other pretreatments, such as thermomechanical extrusion,¹¹ acid hydrolysis and alkaline treatment.¹² Lasmal *et al.*¹³ observed that greater particle-size reduction (PS < 0.132 mm) led to a higher reducing sugar yield of wheat bran and soybean hull after enzymatic hydrolysis for ethanol production. This was due to more available surface area and reduced crystallinity for enzymatic activity at lower particle-size reduction. The combined effect of temperature and shear during extrusion might lead to greater surface area and also to the deconstruction of hemicellulose chains, which might otherwise interfere with enzymatic hydrolysis.¹³ This result was also confirmed by Myat and Ryu,¹¹ who reported that thermomechanical extrusion and greater particle-size reduction (0.3 > PS ≥ 0.15 mm) gave the highest fermentable sugar yield for ethanol production from corn fiber.

Thermomechanical extrusion

Thermomechanical extrusion is a novel and promising physical pretreatment technology for biomass conversion to ethanol production. In extrusion, materials are subjected to heating, mixing, and shearing, resulting in physical and chemical changes during passage through the extruder.¹⁴ Screw speed and barrel temperature have been believed to disrupt the lignocellulose structure, causing fibrillation and shortening of the fiber and increasing the accessibility of carbohydrates to enzymatic attack.¹⁵ It is well known that when material passes through the extruder barrel, high pressure is developed and when the extruded material comes out of the die, it experiences low pressure, thus exploding, which makes cellulose and hemicellulose more amenable to hydrolysis.¹⁶

Extrusion pretreatment can provide a unique reactor environment for a combination of thermomechanical and chemical pretreatment of lignocellulosic biomass.¹³ This alternative pretreatment method was first investigated in the 1980s for the pretreatment of crop residues, sawdust and municipal waste in the presence of dilute sulfuric acid based on a method developed at New York University.^{17,18} Extrusion also changes the content, composition and physiochemical properties of dietary fiber. Fiber content can be lowered due to degradation of dietary fiber into lower molecular weight fragments. Macromolecular degradation of fiber by extrusion increases its solubility and changes its physiological properties.¹⁹

Myat and Ryu²⁰ reported that extrusion pretreatment increased soluble dietary fiber and functional properties such as protein digestibility and free amino nitrogen of corn fiber for yeast nutrient in fermentation. The increase in protein digestibility could be due to protein denaturation, which may increase exposure sites susceptibility to enzymatic activity²¹ and the inactivation of trypsin and chymotrypsin inhibitors, leading to improved digestibility.²² During extrusion, the hydrolysis of destarched corn fiber also might help the release of free amino nitrogen content in the extruded sample,^{23,24} which will favor yeast growth, and increase the ethanol content.²⁰

Therefore, the application of extrusion pretreatment is considered as a promising technology for lignocellulosic ethanol production.

Chemical pretreatments

Acid pretreatment

Concentrated acid has been initially applied to solubilize hemicellulose either in combination with hydrolysis of cellulose to glucose or prior to dilute acid hydrolysis of cellulose. Hemicellulose solubilization increases porosity and improves enzymatic digestibility, maximum enzymatic digestibility usually coinciding with complete hemicelluloses removal.²⁵ It can be performed at high temperature (e.g. 180°C) during short periods of time or lower temperature (e.g. 120°C) for longer retention time (30-90 min). It presents the advantage of solubilising hemicellulose, mainly xylan and also converting solubilised hemicelluloses to fermentable sugars.

Dilute acid pretreatment has been applied to a wide range of feedstocks, including softwood, hardwood, herbaceous crops, agricultural residues, wastepaper and municipal solid waste. Dilute acid hydrolysis is fast and easy to perform, but it is hampered by non-selectivity and by-product formation and has some disadvantages, such as corrosion of materials, neutralization of hydrolysates before fermentation, formation of degradation products and fermentation inhibitors and disposal of neutralization salts. Therefore, the economic feasibility of dilute acid pretreatment might need to be considered.

Alkaline pretreatment

Alkaline pretreatment is one of the major chemical pretreatment technologies by using various bases, including sodium hydroxide.^{26,27,28} Alkaline pretreatment increases the accessibility of enzyme to cellulose and is more effective for lignin and hemicelluloses solubilisation than acid or hydrothermal processes.²⁹ Sodium, potassium and ammonium hydroxides are suitable for alkaline pretreatments. Sodium hydroxide (NaOH) causes swelling, increasing the internal surface of cellulose, the disruption of the lignin structure, and the separation of the structural linkage between lignin and carbohydrates, and decreasing the degree of polymerization and the crystallinity index.³⁰

NaOH has been reported to increase hardwood digestibility⁷ and enzyme accessibility.³¹ Vaccarino *et al.*³² reported the greatest degrading effects were obtained by 1% NaOH solution at 120°C.

Zhao *et al.*³³ reported that alkaline pretreatment could lead to a higher enzymatic conversion ratio of cellulose compared with acid pretreatment. Alkaline pretreatment has also been reported to increase hardwood digestibility by reducing the lignin content.³⁴ The same conclusion was drawn by Myat and Ryu,³⁵ who reported that thermo-mechanical extrusion followed by alkaline pretreatment (0.75% NaOH) significantly increased ethanol yield from destarched corn fiber by decreasing the crystallinity index and the degree of polymerization for enzymatic hydrolysis, and improving protein digestibility and free amino nitrogen for yeast nutrient in fermentation.

A significant disadvantage of alkaline pretreatment is the conversion of alkali into irrecoverable salts and/or the incorporation of salts into the biomass during pretreatment reactions. Therefore, the treatment using a high concentration of alkaline solution becomes a challenging issue and the lowest concentration is important for alkaline pretreatment.

Combination pretreatment

Steam explosion is the most widely employed physicochemical pretreatment for lignocellulosic biomass. It is a hydrothermal pretreatment in which the biomass is subjected to pressurised steam (2 kg/cm^2) for a period of time ranging from seconds to several minutes, and then suddenly depressurised. This pretreatment combines mechanical forces and chemical effects due to the hydrolysis of acetyl groups present in hemicelluloses. The mechanical effects are caused when the pressure is suddenly reduced and fibers are degraded owing to the explosive decompression. In combination with partial hemicellulose hydrolysis and solubilisation, the lignin is redistributed and to some extent removed from the material.³⁶ The removal of hemicelluloses

increases enzyme accessibility to the cellulose microfibrils.

The most important factors affecting the effectiveness of steam explosion are particle size, temperature and residence time. Higher temperature results in an increased removal of hemicellulose from the solid fraction, enhanced cellulose digestibility, promoting higher sugar degradation. The main drawbacks of steam-explosion pretreatment are partial hemicellulose degradation and the generation of some toxic compounds that could affect the fermentation steps.³⁷ Steam explosion has been proposed as an efficient pretreatment of lignocellulosic material and has the advantage that it can be developed on a commercial scale.³⁸ During steam explosion pretreatment, some degradation products are formed in the aqueous portion of the hydrolysate slurry. They may be potential inhibitors for fermentation. In environmentally sustainable ethanol production from lignocellulose, this aqueous fraction should be used as fermentation broth to minimize fresh water requirements and decrease the amount of waste water produced.³⁸ A universal pretreatment process is difficult to envision owing to the diverse nature of different biomass. No perfect pretreatment method has been established for biofuel production from biomass on a commercial scale.³⁸ Therefore, the development of an environmentally friendly pretreatment technology is essential to improve industrial lignocellulosic ethanol production. The modification of the lignocellulosic substrate is achieved through various pretreatment technologies that disrupt the cell wall structure and make it accessible to enzymes. The effect of pretreatments on lignocellulosic biomass is presented in Figure 1.

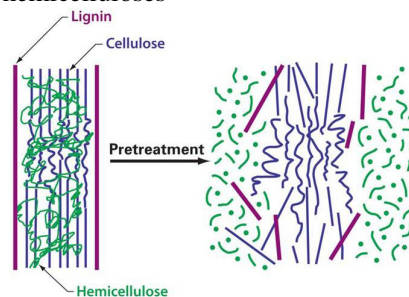


Figure 1: Schematic presentation of pretreatment effects on lignocellulosic biomass³

FACTORS AFFECTING ENZYMATIC HYDROLYSIS (SACCHARIFICATION)

The inherent properties of native lignocellulosic material make it resistant to enzymatic attack. The digestibility of cellulose present in lignocellulosic biomass is hindered by many physicochemical, structural, and compositional factors. When biomass needs to be treated, the cellulose in the biomass is exposed. However the enzymatic hydrolysis of lignocellulose is limited by several factors. Cellulose crystallinity, accessible surface area and protection by lignin and hemicellulose content, and the degree of polymerization are the main factors considered as affecting the rate of enzymatic hydrolysis.³⁹

Cellulose crystallinity

Cellulose crystallinity has been considered as an important factor in determining the hydrolysis rate of cellulosic substrates.² The cellulose microfibrils have both crystalline and amorphous regions, and the crystallinity index is given by the relative amounts of these two regions. Two thirds of the total cellulose is in crystalline form⁴⁰ and it was shown that the cellulase readily hydrolyzes the more accessible amorphous portion of cellulose, while the enzyme is not so effective in degrading the less accessible crystalline portion. A decrease in crystallinity was accompanied by an alteration of other substrate characteristics, such as particle-size reduction or increase in available surface area.⁴¹ It is possible that an improvement of properties in the collapsed state of fiber is accompanied by different effects on the structure of fiber (with different changes in the degree of crystallinity of cellulose in the fiber). Caulfield and Moore⁴² also mentioned that a decrease in particle-size reduction and an increase in the available surface area rather than high crystallinity affect the rate and extent of hydrolysis. It is well known that a low crystallinity index is very important for enzymatic saccharification, along with the interchain hydrogen-bonding network conferring high resistance to enzymatic hydrolysis, whereas amorphous cellulose is readily digestible.⁴³ It is therefore expected that a high crystallinity index will cause resistance to enzymatic hydrolysis and it is widely accepted that decreasing the crystallinity index increases the digestibility of lignocelluloses.⁴⁴ This observation is in agreement with the results of Myat and Ryu,³⁵ who stated that a decrease in the crystallinity index of

extruded destarched corn fiber treated by 0.75% NaOH significantly increased fermentable sugar yield and ethanol yield.

However, an increase in the crystallinity index after the pretreatment of the lignocellulosic substrate using an alkaline solution was observed previously.^{33,35,45,46,47} Lee *et al.*⁴⁸ suggested that some pretreatments would be sufficient for enzymatic saccharification regardless of the crystallinity index. Yoshida *et al.*⁴⁶ also found that delignification increased the rate of the enzymatic hydrolysis of cellulose and hemicelluloses, although biomass showed higher crystallinity index. Kim and Holtzapfle³¹ found that the crystallinity index of corn stover increased through delignification with calcium hydroxide, which was related to the removal of amorphous compounds (lignin and hemicelluloses). Although the crystallinity is an important factor in the digestibility of lignocelluloses, it is not the only factor in an effective enzymatic hydrolysis, due to the heterogeneous nature of cellulose and the contribution of other components such as hemicelluloses.

Recrystallization occurs when cellulose is exposed to high relative humidity,⁴⁹ and prolonged exposure to boiling water sometimes results in crystallization even greater than the original sample.⁵⁰ Tanahashi *et al.*⁵¹ found that the crystallinity index of cellulose in wood increased after high pressure steam explosion due to transformation of cellulose from amorphous to crystalline phase. A decrease in the crystallinity index and an increase in the surface area due to mechanical pretreatment, such as ball milling, can also lead to improved enzymatic saccharification.^{46,52}

Accessible surface area

Several studies have shown a good correlation between the pore volume or population (accessible surface area for cellulose) and enzymatic digestibility of lignocellulosic materials. The main reason for an improvement in enzymatic hydrolysis by removing lignin and hemicellulose is related to the accessible surface area of cellulose. The effect of this area may correlate with the crystallinity index or lignin protection or hemicelluloses presentation or all of them.³⁹ Lignocellulosic materials have external and internal surface area. The external surface area is related to the size and shape of the particles, while the internal surface area depends

on the capillary structure of cellulosic fiber. Swelling of lignocelluloses with water and polar solvents creates large internal surface area.⁴⁴ Parikh *et al.*⁵³ also observed that good swelling media like water and sodium hydroxide solution not only break the H-bonding, but also open up the fiber structure to increase the accessible surface area. Myat and Ryu³⁶ also observed that alkaline pretreatment causes swelling of destarched corn fiber for efficient saccharification and increased ethanol content.

Lignin content

Cellulose and hemicellulose are cemented by lignin. Lignin is responsible for integrity, structural rigidity, and prevention of swelling of lignocelluloses. Lignin limits the rate of enzymatic hydrolysis by acting as a physical barrier, preventing the digestible parts of the substrate to be hydrolyzed.² The reason of an improved rate of hydrolysis by the removal of lignin might be related to a better surface accessibility for enzymes by increasing the population of pores. Therefore, lignin content constitutes the most recognized factor that is responsible for the recalcitrance of lignocellulosic materials to enzymatic degradation by limiting the enzyme accessibility. Esteghlalian *et al.*⁵⁴ and Wyman⁵⁵ also reported that protein binding capacities contained in lignin reduce the total enzyme activity.

Hemicelluloses content

Removal of hemicellulose increases the mean pore size of the substrate, as well as the accessibility and the probability of cellulose to become hydrolyzed.⁵⁶ However, the recovery of sugar from hemicellulose in the pretreated solids would be interesting to obtain higher total fermentable sugar production. The degree of acetylation in the hemicellulose is another important factor because lignin and acetyl groups are attached to the hemicellulose matrix and may hinder polysaccharides breakdown.² Myat and Ryu³⁵ also observed that the removal of hemicellulose from extruded destarched corn fiber significantly increased the xylose yield for fermentation. Typically, cellulose exists as microfibrils, which are sheathed with hemicelluloses and aligned in the direction of the cell walls.

Degree of polymerization

A decrease in the degree of polymerization is also an important factor promoting the digestibility of cellulose during enzymatic hydrolysis.^{2,57} The degree of polymerization (DP) is related to cellulose crystallinity and depends on the nature of the cellulose substrate. In enzymatic hydrolysis, endoglucanases cut the internal sites of the cellulose chains and are primarily responsible for decreasing the degree of polymerization of the cellulose substrates.⁴¹ It was clearly indicated that thermomechanical extrusion was able to depolymerize cellulose by reducing corn fiber molecules into low molecular weight materials, making them more amenable for enzymatic hydrolysis.^{35,58} This would lead to greater exposure of cellulose to the enzymatic action and to a more efficient saccharification process. The effect of different pretreatments on cellulose chain length has been studied. Reducing the DP had a more severe impact on the cellulose chain length.⁷ Wang *et al.*⁵⁹ stated that the lowest DP value (1.92) resulted for the highest reducing sugar yield of rice straw by feed xylanase with ultrafiltration. Myat and Ryu³⁵ also observed that the lowest DP value of destarched corn fiber significantly increased fermentable sugar yield for ethanol production. It is reported that the DP has been considered as an important factor in determining the hydrolysis rates of cellulosic substrates.⁶⁰ The effects of different pretreatments on the physicochemical properties of lignocellulosic biomass for enzymatic saccharification were presented in Table 1.

FACTORS AFFECTING FERMENTATION

The pretreatment of lignocellulosic biomass may produce degradation products with an inhibitory effect on fermentation process. These inhibitors have toxic effects on fermenting organisms, thus reducing the ethanol yield and productivity. The major types of inhibitors are phenolic compounds, furfural, 5-hydroxymethylfurfural (HMF), acetic acid and extractives. The relative toxicity of the various inhibitors for ethanol fermentation can be summarized as following the order: phenolic compounds > furfural > HMF > acetic acid > extractives.

Sugar degradation products

Subsequent to hemicellulose hydrolysis, pentose may degrade into inhibitors such as furfural.

Similarly, hexose (glucose) may degrade to toxic 5-hydroxymethylfurfural (HMF). HMF is considered less toxic than furfural and its concentration in hemicellulose hydrolysate is usually low. The effect of furfural on the cultivation of yeast has been considered in many studies. A significant decrease in the furfural content of extruded destarched corn fiber significantly increased the ethanol content.⁶³ Kinetic studies have shown that the production of furfural strongly increases with temperature and reaction time. Temperatures higher than 160°C and residence time of acid pretreatment longer than 4 h have been reported to lead to the formation of furfural or HMF.⁶⁷ The formation of furfural and HMF should be prevented by keeping the process temperature and residence time as low and as short as possible.

Lignin degradation products

A variety of compounds (e.g. aromatic, polyaromatic, phenolic and aldehydic) may be released from the lignin fraction. Phenolic compounds have been suggested to exert a considerable inhibitory effect in the fermentation of lignocellulosic hydrolysates⁶⁸ and are more toxic than furfural and HMF.⁶⁹ Phenolic compounds cause partition and loss of integrity of the cell membranes of the fermenting organisms reducing cell growth and sugar assimilation. This observation was also confirmed by Myat and Ryu,¹¹ who observed that a significant decrease in total phenolic content of corn fiber after thermomechanical extrusion and particle-size reduction significantly increased ethanol content of corn fiber. The reduction in total phenolic content may be attributed to the alteration in the molecular structure of phenolic compounds, which may lead to a reduction in the chemical reactivity.⁷⁰ The main factors affecting the formation of lignin degradation products are process temperature and residence time. At a temperature lower than 180°C, lignin degradation is negligible, if no strong acid or alkaline conditions are present.

The minimum amount of toxic compounds after the pretreatment is one of the key factors to take into consideration for an effective, low-cost and advanced pretreatment process.⁵⁵ A pretreatment may lead to the generation of toxic compounds derived from sugar decomposition that could affect the subsequent hydrolysis and fermentation steps.³⁷ It has also been reported that

important changes in the phenolic content caused by pretreatments might produce adverse effects in human and animal nutrition.⁷¹

Acetic acid and extractives

Acetic acid is derived from the acetyl groups in hemicellulose. The inhibitory effect of acetic acid is pH dependent since it is undissociated acetic acid, which penetrates the cell membrane and dissociates intracellularly due to the higher intracellular pH. At low pH in the fermentation medium, acetic acid diffuses into the cells, causing a lowering of cell pH, which inhibits cell activity. However, fermentation at higher pH (pH 5.5) can reduce this effect or the acid can be neutralized before fermentation. Extractives are derived from the lignocellulose structure and include acidic resins, tannic and terpene acids. These extractives are less toxic than lignin breakdown products or acetic acid.

Protein digestibility

Protein digestibility has been used as a quality indicator of protein availability and the susceptibility of a protein to proteolysis. The factors that contribute to poor protein digestibility may be divided into two categories. Exogenous factors are factors that arise out of the interaction of protein with non-protein components like polyphenols, non-starch polysaccharides, starch, phytates and lipids. Endogenous factors refer to the factors that arise out of the changes within the protein themselves and do not involve interaction of the protein with non-protein components.⁷² During pretreatment, protein may interact with non-protein components and the protein themselves may undergo changes.⁷³ Most proteins undergo structural unfolding and/or aggregation when subjected to heat or shear. Denaturation of protein may actually lead to improvement in protein digestibility.⁷⁴

Protein digestibility has been used as a nutritional indicator for fermentation because yeast cannot produce any exoprotease like humans and animals during fermentation. Additionally, protein nutritional value is mainly dependent on the digestibility. Wang *et al.*⁷⁵ reported a strong linear correlation between protein digestibility of grain sorghum and fermentation efficiency in ethanol production.

Table 1
Effects of pretreatments on physiochemical properties of lignocellulosic biomass for enzymatic saccharification

Biomass	Pretreatment method	Pretreatment effects	References
Corn fiber	Thermomechanical extrusion and particle-size reduction (0.5 > PS ≥ 0.3)	Decreased lignin and crystallinity index	Myat and Ryu ¹¹
Wheat bran and soybean hull	Particle-size reduction (PS < 0.132 mm)	Increased surface area and decreased crystallinity index	Lasmalet <i>et al.</i> ¹³
Crop residues, sawdust, municipal waste	Thermomechanical extrusion	Decreased crystallinity index	Noon and Hochstelter, ¹⁷ Green ¹⁸
Destarched corn fiber	Thermomechanical extrusion	Decreased lignin, degree of polymerization and crystallinity index	Myat and Ryu ²⁰
Solid residue of olive, wheat straw and cotton stalk	Alkaline	Delignification, saponification and increased accessibility	Abdi <i>et al.</i> , ²⁶ Carrilo <i>et al.</i> , ²⁷ Silverstein <i>et al.</i> ²⁸
Destarched corn fiber	Thermomechanical extrusion and 0.75%NaOH	Decreased lignin, degree of polymerization	Lue <i>et al.</i> , ¹⁹ Myat and Ryu ³⁵
Corn stover	Alkaline twin-screw extrusion	Increased delignification and specific surface area	Liu <i>et al.</i> ⁶¹
Wheat straw	Dilute acid (0.75% H ₂ SO ₄)	Hemicellulose converted to fermentable sugar	Saha <i>et al.</i> , ⁶² Chen <i>et al.</i> ²⁵
Destarched corn fiber	0.75%H ₂ SO ₄	Decreased hemicellulose	Myat and Ryu ⁸⁶
Destarched corn fiber	0.75%NaOH	Decreased hemicellulose and lignin	Myat and Ryu ⁸⁶

The increase in protein digestibility may be caused by protein denaturation, which may increase exposure sites susceptible to enzymatic activity²¹ and inactivation of trypsin and chymotrypsin inhibitors, leading to improved digestibility.²² The higher susceptibility of protein to pepsin, the more antitrypsin activity in the substrate. This observation was also confirmed by Myat and Ryu,²⁰ who found that a significant increase in protein digestibility of extruded destarched corn fiber significantly increased the ethanol content. A protein with high digestibility potentially has better nutritional value than those with low digestibility, because it would provide more amino acids for absorption on proteolysis.

Free amino nitrogen

The availability of yeast food is vital to yeast growth and its efficiency in converting fermentable sugars into ethanol during fermentation. Most yeast fermentation systems need nutrient supplementation. Yeast uptakes not only fermentable sugars for ethanol production, but also nutrients (amino acids, minerals, and vitamins) for its own growth and functional maintenance, which are responsible for sugar transportation and conversion. Free amino nitrogen (FAN) is an essential nutrient for yeast growth during fermentation^{76,77} and is required as a yeast nitrogen source for rapid and complete fermentation.^{76,78} Because amino acids are chemically diverse molecules, the most convenient measure of assimilable nitrogen related to assaying free or α -amino group of the primary amino acids is free amino nitrogen (FAN).

It is known that the nitrogen level in a medium containing glucose as carbon source can be adjusted to give an increased ethanol production during fermentation by *Saccharomyces*. It has been suggested recently that nitrogen stimulates glycolysis in yeast indirectly through its requirement for protein synthesis.⁷⁸ Mullins and NeSmith⁷⁹ also

observed that the addition of nitrogen, 300 mg nitrogen/L, gave the expected increase in the rate of ethanol production. When a nitrogen source is supplemented in the fermentation system, the nutritional supplement can promote rapid fermentation to a higher ethanol level.⁸⁰ Fermentation requires an adequate supply of nitrogenous compounds to yeast growth and proliferation. Therefore, the FAN content in a fermenting substrate sample could be a useful indicator of the sample's performance in ethanol fermentation.

One of the factors limiting the production of high ethanol content by brewing yeast is nutritional deficiency.⁸¹ The higher the FAN content in the fermented slurry, the faster the fermentation process. Mullins and NeSmith⁸⁰ studied ethanol fermentation with high-tannin sorghum and revealed that the addition of nitrogen accelerated the ethanol fermentation rate. Yan *et al.*⁸² drew the same conclusion about the effect of FAN on the fermentation efficiency of field-sprouted sorghum and wheat. The assimilable nitrogen was found to be related to FAN, and the addition of diammonium phosphate (DAP) increased the fermentation rate.⁸³ Hydrolysis of biomass might help the release of FAN content,^{23,24} which will favor yeast growth, and increase the ethanol content. FAN in the fermenting substrate is important for yeast performance and *Saccharomyces cerevisiae* is capable of only assimilating amino acids. Myat and Ryu³⁵ also observed that a significant increase in the FAN content of extruded destarched corn fiber significantly increased the ethanol content after fermentation. On the other hand, nitrogen deficiencies have been reported as a major cause for slow fermentation.^{84,85} Therefore, an adequate level of free amino nitrogen is required as a yeast nitrogen source for rapid and complete fermentation.⁷⁶ The effects of pretreatments on fermentation inhibitors for yeasts during fermentation were listed in Table 2.

Table 2
Effects of pretreatments on fermentation inhibitors for yeasts during fermentation

Biomass	Pretreatment	Pretreatment effects	References
Corn fiber	Thermomechanical extrusion and particle-size reduction	Decreased total phenolic content	Myat and Ryu ¹¹

Sorghum	Supercritical fluid extrusion	Decreased total phenolic content	Zhan <i>et al.</i> ¹⁴
Destarched corn fiber	Thermomechanical extrusion	Increased protein digestibility and free amino nitrogen	Myat and Ryu, ²⁰ Thomas and Ingledew, ²³ Perez-Carrillo <i>et al.</i> ²⁴
Sorghum	Thermomechanical extrusion	Depolymerization of dietary fiber and destruction of starch and protein matrix	Camire ⁶⁴
Destarched corn fiber	Thermomechanical extrusion and 0.75% NaOH	Decreased furfural content	Myat and Ryu ⁶⁵

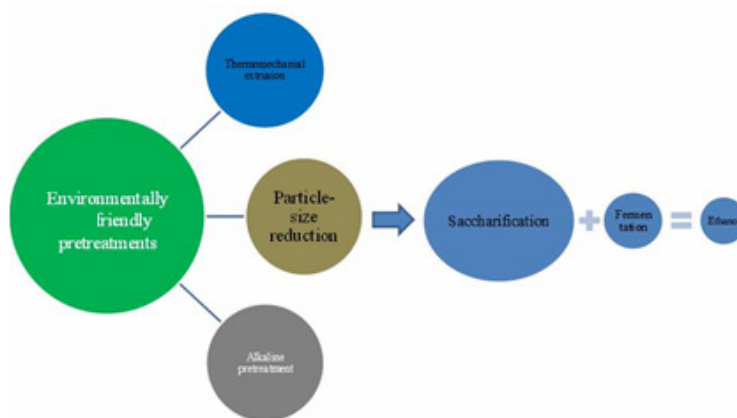


Figure 2: Presentation of environmentally friendly pretreatments for lignocellulosic ethanol production

CONCLUSION AND PERSPECTIVES

Different pretreatment technologies reported in a number of scientific journals were described in terms of mechanisms involved, advantages and disadvantages. The choice of the optimum pretreatment technologies depends on the objective of biomass pretreatments and kind of biomass materials, since different products are yielded during pretreatment. However, based on the factors affecting enzymatic saccharification and fermentation inhibitors, thermomechanical extrusion is a potential pretreatment method to combine with other pretreatments, such as particle-size reduction and alkaline (NaOH) pretreatment for lignocellulosic ethanol production.

The factors affecting saccharification and fermentation have been described to improve enzymatic hydrolysis in ethanol production. Moreover, cellulose crystallinity, lignin and hemicellulose contents, and the degree of polymerization are the main factors affecting the enzymatic hydrolysis of lignocellulosic

biomass. Finally, total phenolic content and furfural content affect yeast growth during fermentation. Protein digestibility and free amino nitrogen are also the main nutrients for yeast in fermentation.

Recently developed pretreatment technologies based on thermomechanical extrusion, particle-size reduction and alkaline (NaOH) pretreatment leading to delignification, increase the accessible surface area and decrease the crystallinity index and the degree of polymerization for efficient enzymatic saccharification of lignocellulosic biomass. A decrease in total phenolic and furfural contents and an increase in protein digestibility and free amino nitrogen are additional benefits of thermomechanical extrusion for yeast growth and nutrient during fermentation. The present review paper investigated the optimization of an environmentally friendly pretreatment for future lignocellulosic ethanol production. Based on the results observed in previous research,^{11,20,36,66} combined pretreatments such

as thermomechanical extrusion, particle-size reduction and alkaline pretreatment (NaOH) should be applied to improve industrial lignocellulosic ethanol production (Fig. 2).

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