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# Different Pretreatment Methods of Lignocellulosic Biomass for Use in Biofuel Production

*Muhammad Nauman Aftab, Irfana Iqbal, Fatima Riaz, Ahmet Karadag and Meisam Tabatabaei*

## Abstract

Lignocellulosic biomasses are carbon neutral and abundantly available renewable bioresource material available on earth. However, the main problem that hinders its frequent use is the tight bonding within its constituents that include cellulose, hemicellulose, and lignin. The selection of pretreatment process depends exclusively on the application. Various pretreatment processes are primarily developed and utilized in effective separation of these interlinked components to take maximum benefit from the constituents of the lignocellulosic biomasses especially for the production of biofuel. The major pretreatment methods include physical, chemical, thermophysical, thermochemical, and biological approaches. Various aspects of these different pretreatment approaches are discussed in this chapter.

**Keywords:** pretreatment methods, lignocellulosic biomass, biofuel production

## 1. Introduction

We are living in a world of many challenges such as climate changes, polluted environment, resource depletion, and increasing demand for fuel. The use of oil reserves to fulfill our need of fuel has caused many drastic challenges from energy security to change in temperature. Rapid industrialization has increased the demand of petroleum products and consequently has raised the monopoly of few countries, which can manipulate petroleum price and create instability. This may also create environmental problems by emission of greenhouse gases and subsequently effect on climate change. The most important source of energy is petroleum that is largely used in transportation and industries; therefore, viability of liquid fuel is enhanced. As the environmental issues are growing, more research is being conducted to address the problems. The search for alternative source of petrol that is less costly with minimal environmental effects has become the center of attention. For instance, biomass is considered as a sustainable resource that can be utilized in large-scale production of biofuel that can be utilized as an alternative source of fuel and may present solution to environmental problems. Furthermore, relying on fossil fuel could be detrimental as it has been predicted of its depletion by 2050. The total annual primary production of biomass is over 100 billion tonnes of carbon per year, and the energy reserve per metric tonne of biomass is between 1.5E3 and 3E3 kW hours that is sufficient to cater the needs of the world energy requirements [1].

Bioenergy products like bioethanol, biohydrogen, and biodiesel can be obtained from lignocellulose biomass which is considerably large renewable bioresource and obtained from plants. The term “lignocellulosic biomass” is defined as lignin, cellulose, and hemicellulose that constitute the plant cell wall. Strong cross-linking associations are present between these components that cause hindrance in the breakdown of plant cell wall. Polysaccharides and lignin are cross-linked via ester and ether linkages [2–4]. Microfibrils that are formed by cellulose, hemicellulose, and lignin help in the stability of plant cell wall structure [5, 6].

Lignocellulose was first produced from food crop such as corn, oilseed, and sugarcane. But the use of edible feedstock for bioenergy products formation is being discouraged to prevent the rise in food competition. Thus, second-generation biofuels are obtained from plants wastes to avoid competition of land and water resources between energy crops and food crops. Currently, lignocellulose is being produced from wood residues, agricultural residues, food industry residue, grasses, domestic wastes, municipal solid wastes, and nonfood seeds [7–9]. The lignocellulose wastes (LCW) are largest renewable bioresource reservoir on earth that is being wasted as pre and postharvest agricultural wastes. Thus, many steps need to be adopted for use of these renewable resources for the production of bioenergy products. Recovery of many products like enzymes, methane, activated carbon, lipids, resins, methane, carbohydrates, surfactants, resins, organic acids, ethanol, amino acids, degradable plastic composites, biosorbents, biopesticides, and biopromoters can be achieved by utilizing LCW. The added benefits of using LCW besides recovery of different products are the removal of LCW waste from the environment. Also, utilization of LCW eliminates the use of food for bioethanol production. The US government has planned the production of 21 billion gallon of biofuels by 2022 [2, 5]. Biofuel production from lignocellulosic biomass reduces the emission of greenhouse gases.

Pretreatment brings physical, biological, and chemical changes to biomass structure; therefore, it is very important to consider the type of pretreatment. In order to break down the hindrance caused by strong association within the cell wall, pretreatment is an important step which can increase the availability of lignocellulosic biomass for cellulase enzymes, their digestibility, and product yield. Before subsection to enzymatic hydrolysis, pretreatment of biomass can increase the rate of hydrolysis by 3–10-fold. Pretreatment of LCW is not an easy step as it seems after the installation of power generator; pretreatment is the second most costly process at industrial level. In crystalline cellulose, the disruption of hydrogen bonds, cross-linked matrix disruption, and increase in porosity as well as surface area of cellulose are the three tasks that are performed via a suitable pretreatment methods. The outcome of pretreatment also differs due to the difference in the ratio of cell wall components [10, 11]. The option to use dilute acid pretreatment method is more effective against poplar tree bark or corn as compared to the same method used for sweet gum bark or cornstalks. Few requirements of an effective, efficient, and economically suitable pretreatment process that including use of cheap chemicals, very less consumption of chemicals, prevention of hemicellulose and cellulose from denaturation, minimal energy requirement and consumption, cost-effective size reduction process, and reactive cellulosic fiber production are the factors that need to be considered for pretreatment. There are several methods of pretreatment that can be divided into four categories, namely, chemical, physical, biological, and physiochemical pretreatment [12–15].

## **2. Physical methods**

Pore size and surface area of lignocellulosic biomass can be increased, whereas crystallinity and degree of polymerization of cellulose can be decreased with the

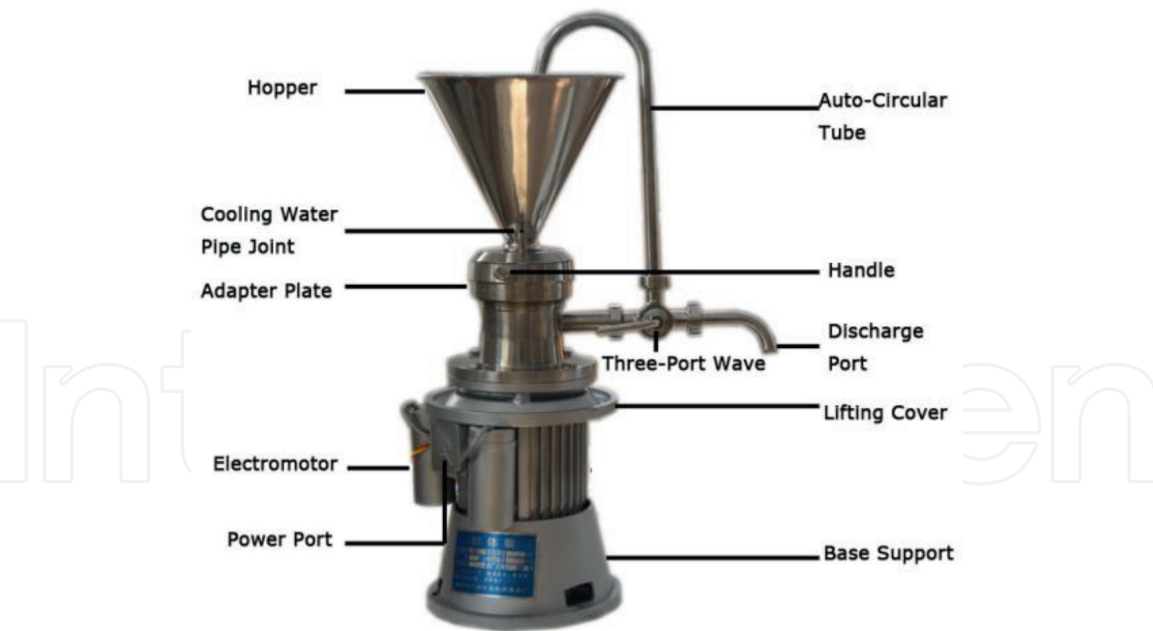
application of physical methods. Physical pretreatments include milling, sonication, mechanical extrusion, ozonolysis, and pyrolysis.

## 2.1 Milling

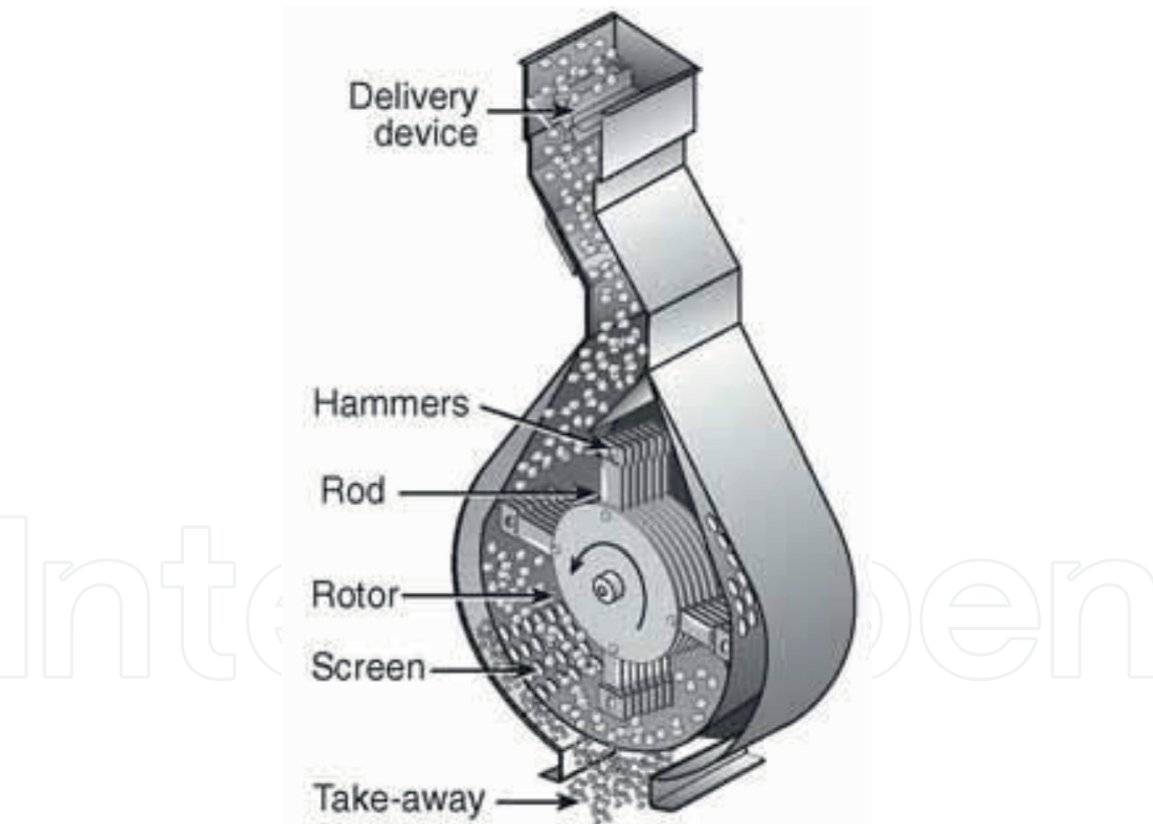
On the inherent ultrastructure of cellulose and degree of crystallinity, milling can be performed to render lignocelluloses more amenable to cellulases. Cellulases are enzyme that catalyze cellulose, but for the catalysis and best results, the substrate availability needs to be enhanced for optimized functioning of the enzymes. Before the subjection of the LCW to enzymatic hydrolysis, milling and size reduction of the lignocellulosic matter should be performed. Milling process has several types like ball milling, colloid milling, vibro-energy milling, hammer milling, and two-roll milling. For wet material, colloid mill, dissolver, and fibrillator are suitable, whereas for dry materials hammer mill, extruder, cryogenic mill, and roller mill are used. For both wet and dry material, ball milling can be used. For waster paper, hammer milling is the most suitable pretreatment option. Enzymatic degradation can be improved by milling as it reduces the degree of crystallinity and material size. Up to 0.2 mm reduction in particle size can be seen by milling and grinding. Reduction in particle size of biomass can be achieved up to a certain limit; beyond that limit reduction in particle size does not effect in the pretreatment procedure. Corn stover with small particle size, i.e., from 53 to 75  $\mu\text{m}$ , is more productive as compared to large particle size corn stover ranging from 475 to 710  $\mu\text{m}$ . The difference in particle size shows that productivity can significantly affect the pretreatment process. Ball milling causes a massive drop in crystallinity index from 4.9 to 74.2% which makes this process more suitable for saccharification of straw at mild hydrolytic conditions with more production of fermentable sugars [12, 16–18]. For better results of hydrolysis, milling can be used in combination with enzymatic hydrolysis. Mechanical action, mass transfer, and enzymatic hydrolysis can be achieved at the same time when two methods are combined. A number of ball beads in bill mill reactor play a crucial role in the  $\alpha$ -cellulose hydrolysis, as less enzyme loading is required, and 100% rate of hydrolysis can be achieved in comparison to pretreatment of biomass that is carried without the use of milling procedure. Highest hydrolysis rate with high yield of reducing sugar was obtained when rice straw was put into fluidized bed opposed jet mill for fine grinding after cutting, steam explosion, and pulverization. For pretreatment of biomass, ball milling is an expensive option in terms of energy consumption, which is a huge disadvantage at industrial scale. Also, incapability of milling for removing lignin makes it a less suitable option as enzyme accessibility to the substrate is reduced in the presence of lignin. Reduction in crystallinity, degree of polymerization, and increase in surface area can be effected by the type of biomass, type of milling used for pretreatment, and duration of the milling process [19–21].

For improving digestibility and reducing crystallinity, vibratory ball milling is very effective. Low energy consumption has an important advantage of using wet disk milling which produces fibers that improve hydrolysis of cellulose, whereas hammer milling produces finer bundles. Due to this reason milling is not preferred when wet disk milling is available [22, 23]. Other study results of conventional ball and disk milling are compared. With the use of conventional ball milling, maximum yields of xylose and glucose were obtained, i.e., 54.3 and 89.4%, respectively [24]. Wet milling produces less yield, but it has the advantage of not producing inhibitors and very low energy consuming capability. An increase of 110% in enzymatic hydrolysis was achieved when wet milling was combined with alkaline pretreatment. Optimum parameters for wet milling pretreatment of corn stover were 10 mm diameter 20 steel balls, 1:10 solid-to-liquid ratio, 350 rpm/min speed, and 0.5 mm particle size [25] (**Figures 1 and 2**).





**Figure 1.**  
*Colloid milling (Pharmapproach.com).*



**Figure 2.**  
*Hammer milling (Solidswiki.com).*

## 2.2 Microwave

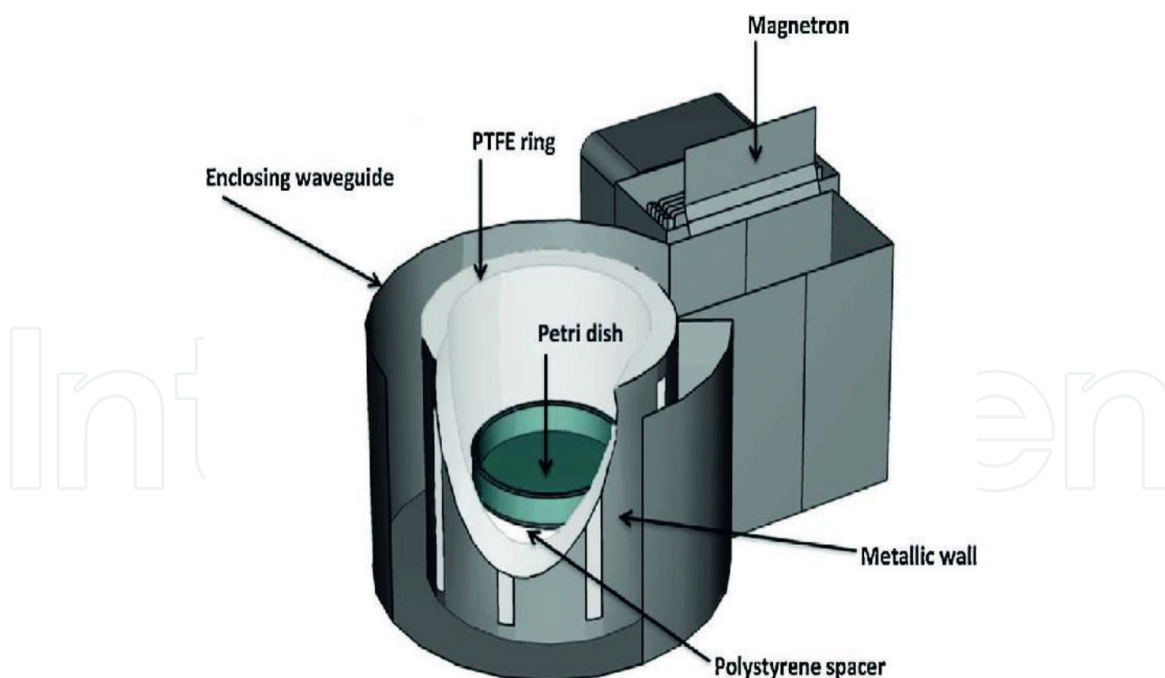
Commonly used method for plant biomass pretreatment is microwave irradiation. This pretreatment method has several advantages that include ease of pretreatment, increased heating capacity, short processing time, minimal generation of inhibitors, and less energy requirement. Microwave irradiation in closed container was first reported in 1984 by team of researchers from Kyoto University,

Japan. They treated sugarcane bagasse, rice straw, and rice hulls with microwaves in the presence of water. The conditions used for microwave treatment include glass vessels of 50 mL, 2450 MHz energy, and 2.4 kW microwave irradiation [26]. Classical pretreatment methods were carried out at high pressure and temperatures. Chemical interactions between lignocellulosic material break as a result of high temperature, thus increasing substrate availability to the enzymes. Under high-pressure steam injection or indirect heat injection, high temperature between 160 and 250°C is provided to lignocellulosic material in conventional heating methods. However, in order to prevent temperature gradients, crushing of lignocellulosic material into small particles is needed. To avoid large temperature gradients, microwave is a good choice as it uniformly distributes heat which also avoids degradation of lignocellulosic material into humic acid and furfural. For effective degradation, microwave irradiation is combined with mild alkali treatment. Sugar yield of 70–90% from switch grass was obtained from alkali and irradiation combined pretreatment [27]. As microwave irradiation is performed at high temperature, therefore, closed containers are required to achieve high temperature. Three properties, namely, penetration, reflection, and absorbance are exhibited by microwave. Microwave passes through glass and plastic, absorbed by water and biomass, whereas microwaves are reflected by metals. Based on these properties, microwave reactors can be divided into two types, one that allows the passage of microwaves, whereas the other kind reflects the microwaves. Glass or plastic is the building material of the first type of microwave reactors, whereas the second types of reactors are composed of steel. Through quartz windows, microwaves can enter into the reactor as these are placed in the reactor. Closed, sealable, pressure-resistant glass tube container having gasket made up of Teflon can be used for the high temperature, i.e., 200°C, for microwave irradiation pretreatment. Sensors are used to control and ensure temperature inside the microwave. Teflon-coated sensors are a good choice because of the thermostability, corrosion-free nature, and zero absorbance properties. In a microwave oven, Teflon vessels are used by some scientists due to its advantageous properties [28, 29]. Normally vessel sizes vary from 100 mL to several hundred milliliters. A 650 mL vessel with 318 mm length, connected nitrogen bottle, gauges, and thermometers are installed on the top of the microwave that was designed by Chen and Cheng [30]. Besides the glass vessels and stainless steel tanks with temperature and pressure sensors, automatic controlling system for microwave input and mechanical stirrer are also used (**Figure 3**).

### 2.3 Mechanical extrusion

When materials that can pass through a defined cross section die, it appears out with the fixed definite profile. This is the extrusion process which is known for sugar recovery from biomass. Adaptability to modifications, no degradation products, controllable environment, and high throughput are few advantages related to mechanical extrusion pretreatment process. Single screw extruder and twin screw extruder are two types of extruders.

Single screw extruder is based on three screw elements, forward, kneading, and reverse. With the minimum shearing and mixing, bulk material of varying pitches and lengths can be transported by forward screw element. Prominent mixing and shearing effect is produced by kneading screw elements with weak forward conveying effect, whereas the use of immense mixing and shearing involves material that is pushed back by reverse screw elements. A screw configuration is defined by the arrangement of different stagger angles, lengths spacing, pitches, and positions. Twin screw extruder can accomplish multiple tasks at the same time like mixing, shearing, grinding, reaction, drying, and separation. High enzymatic

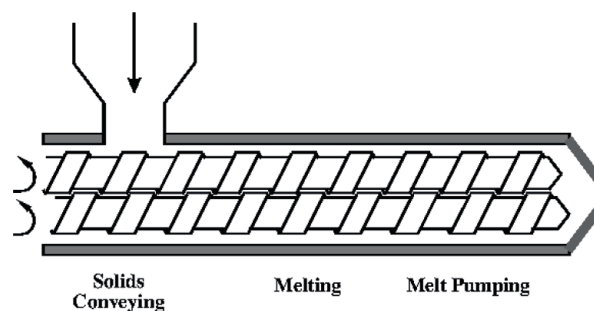


**Figure 3.**  
Microwave irradiation (Researchgate.net).

hydrolysis rates are achieved by the use of single and twin screw extruders. Different parameters like speed of screw, temperature of barrel, and compression ratio can significantly affect recovery of sugars. Short-time extruders provide fast heat transfer, proper mixing, and increased shear. When material passed through the extruder barrel, structure of biomass is disturbed, exposing more surface for enzymatic hydrolysis [31–33]. During extrusion process, lignocellulosic material can be treated with alkali or acid in order to increase sugar recovery. Acidic treatment is less preferred than alkali because of the corrosion caused by acid to the extruder material. Corrosion problem can be solved by the use of AL6XN alloy for barrel fabrication and screws of extruder. With less carbohydrate degradation and role in the delignification, alkali treatment is suitable for lignocellulosic material. Sodium hydroxide is most commonly used to break ester linkages and solubilization of lignins and hemicelluloses. Alkali treatment can be applied by addition of alkali using volumetric pump into the extruder or by soaking the lignocellulosic material in alkali at room temperature [31, 34, 35] (**Figure 4**).

## 2.4 Pyrolysis

For the production of bio oil from biomass, process of pyrolysis is used. Pyrolysis is a thermal degradation of lignocellulosic biomass at very high temperature without the presence of oxidizing agent. At temperature ranging between 500 and 800°C, pyrolysis was performed. Rapid decomposition of cellulose resulted in the formation of products like pyrolysis oil and charcoal [36]. Based on temperature, pyrolysis pretreatment process is divided into fast and low pyrolysis. Certain factors affect the end products like biomass characteristics, reaction parameters, and type of pyrolysis. Due to high-value energy-rich product formation, easy transport management retrofitting, combustion, storage, and flexibility in utilization and marketing, thermal industries are adapting to the process of pyrolysis. Presence of oxygen and less temperature increase the efficiency of this process. A study on the bond cleavage rate of cellulose was carried out in the presence of nitrogen and oxygen. During the process of pyrolysis, breakage of  $7.8 \times 10^9$  bonds/min/g cellulose



**Figure 4.**  
 Twin screw extruder (Researchgate.net).

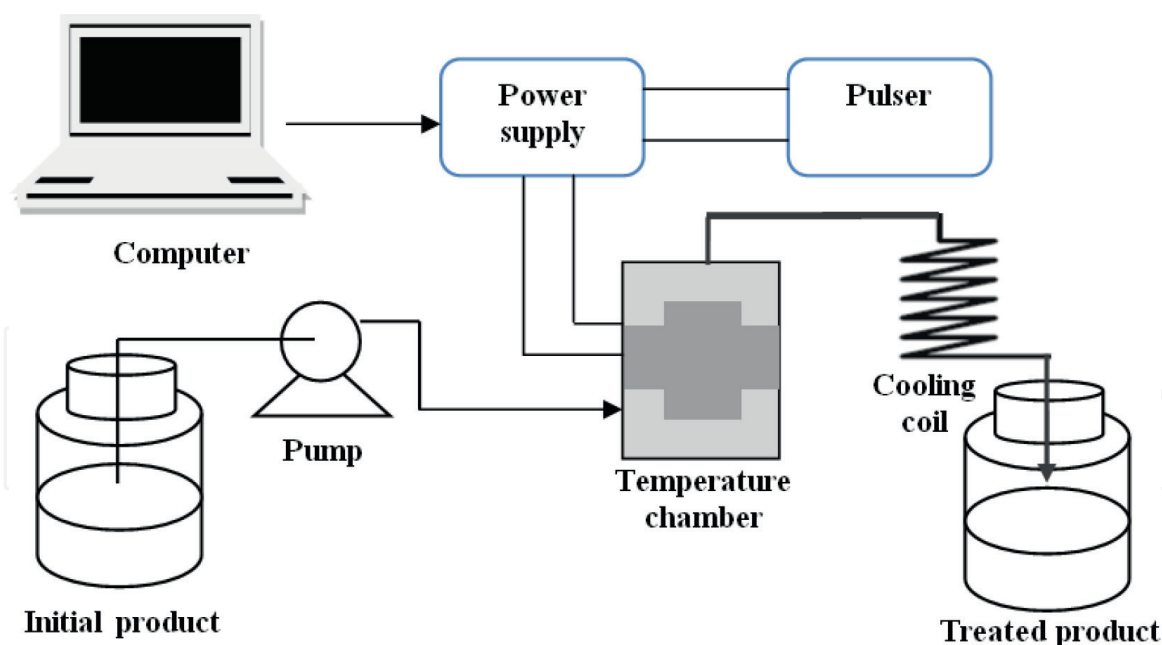
in the presence of oxygen and breakdown of  $1.7 \times 10^8$  bonds/min/g cellulose in the presence of nitrogen at 25°C were observed. In order to obtain more efficiency and results, microwave-assisted pyrolysis is preferred due to the microwave dielectric heating [37]. Thermochemical conversion of biomass into biofuels can be performed via three technologies, gasification, pyrolysis, and direct combustion [38]. Different yields of products from pyrolysis are due to different modes of pyrolysis. Bio oil is a mixture of polar organics and water. Pyrolysis is used where bio oil production is required. Fast pyrolysis in a controlled environment leads to the formation of liquid products (fuels). Torrefaction is an emerging technique which is also known as mild pyrolysis. It differs from pyrolysis with reference to thermochemical process that is carried out at temperature range between 200 and 300°C. Partial decomposition of biomass occurs in this process, and ultimate product obtained is torrefied biomass. Whereas, in the process of pyrolysis, plant biomass is decomposed into vapor, aerosols, and char. Torrefaction has been categorized into two categories based on dry and wet torrefaction.

Dry torrefaction needs an inert environment and completely dry biomass and normal atmospheric pressure. Biochar is the major product in this type of biomass pretreatment. Hydrothermal carbonization and hydrothermal torrefaction are other terminologies used for wet torrefaction. Unlike dry torrefaction, pressurized vessel of water is used to carry out the pretreatment. Biomass used for wet torrefaction contains moisture content, but after torrefaction, a drying process is necessary in this type of torrefaction. A pressure between 1 and 250 MPa is required to carry out wet torrefaction. Biomass used during wet torrefaction pretreatment produces hydro-char as a main product [39].

## 2.5 Pulse electric field (PEF)

In this method, pores are created in the cell membrane due to which cellulose exposes to such agents that cause its breakdown by entering into the cell. High voltage ranging between 5.0 and 20.0 kV/cm is applied in a sudden burst to biomass for nano- to milliseconds. Sample was placed between two parallel plate electrodes, and the strength of electric field is given as  $E = V/d$ , where  $V$  and  $d$  are voltage and distance, respectively, between plate electrodes. Dramatic increase in mass permeability and tissue rupture occurred on the application of electric field. Electric pulses are applied, generally in the form of square waves or exponential decay. Setup of pulse electric field consisted of pulse generator, control system, data acquisition system, and material handling equipment [40, 41]. At ambient temperatures, the treatment can be performed at low energy. Another advantage of this treatment is the simple design of the instrument. Short duration of pulse time saves the effort and energy [42, 43]. Pulse electric field pretreatment was applied to pig manure and waste activated sludge by Author et al. [44]. As compared to untreated





**Figure 5.**  
Pulse electric field (Intechopen.com).

manure and sludge, 80% methane from manure and twofold increase in methane production from sludge were recorded in the study. A PEF system was designed and developed by Kumar et al. [45] that consisted of high-voltage power supply, switch circuit, a function generator, and sample holder. Neutral red dye was used to study the changes in the structure of cellulose by PEF pretreatment. Function generator drives the transistor present in the switching circuit; when pulse is applied by function generator to the switching circuit, switching circuit is turned on. Switching circuit is then transferred to the high voltage across the sample holder. So, by using function generator pulses of desired shape, width and high voltage can be applied to the sample. By using this setup, effects were observed on switch grass and wood. Results showed that at  $\geq 8$  kV/cm, switch grass showed high neutral red uptake. At low field strength, structural changes are less likely to occur. Electroporation through pulsed electric field is greatly affected by two parameters, pulse duration and electric field strength. Irreversible electroporation at  $>4$  kV/cm with pulse duration in millisecond and  $\geq 10$  kV/cm with microsecond pulse duration was observed in *Chlorella vulgaris* which showed that pulse duration with a difference in micro- and milliseconds range can effect electroporation. Pulse electric field can increase hydrolysis rate by exposing cellulose to catalytic agents [40, 41, 46] (Figure 5).

### 3. Chemical pretreatments

#### 3.1 Acid pretreatment

In this pretreatment, acids are used to pretreat lignocellulosic biomass. The generation of inhibitory products in the acid pretreatment renders it less attractive for pretreatment option. Furfurals, aldehydes, 5-hydroxymethylfurfural, and phenolic acids are the inhibitory compounds that are generated in huge amount in acid pretreatment. There are two types of acid treatments based on the type of end application. One treatment type is of short duration, i.e., 1–5 min, but high temperature  $> 180^{\circ}\text{C}$  is used, and the second treatment type is of long duration, i.e., 30–90 min, and low temperature  $< 120^{\circ}\text{C}$  is utilized. Due to hydrolysis by acid

treatment, separate step of hydrolysis of biomass can be skipped, but to remove acid, washing is required before the fermentation of sugars [43, 47]. For acid pretreatment, such reactors are required that show resistance to corrosive, hazardous, and toxic acids; therefore, acid pretreatment is very expensive. Flow through, percolation, shrinking-bed, counter current reactor, batch, plug flow are different types of reactors that have been developed. For enhancing economic feasibility of acid pretreatment, recovery of concentrated acid at the end of the treatment is an important step.

To treat lignocellulosic biomass, concentrated acids are also used. Most commonly used acids are sulfuric acid or hydrochloric acid. In order to improve the process of hydrolysis for releasing fermentable sugars from lignocellulosic biomass, acid pretreatment can be given. For poplar, switch grass, spruce, and corn stover, sulfuric acid pretreatment is commonly used. Reducing sugars of 19.71 and 22.93% were produced as a result of the acid pretreatment of Bermuda grass and rye, respectively. In percolation reactor, pretreatment of rice straw was carried out in two stages using aqueous ammonia and dilute sulfuric acid. When ammonia is used, 96.9% reducing sugar yield was obtained, while 90.8% yield was obtained in case of utilization of dilute acid. *Eulaliopsis binata* is a perennial grass and yielded 21.02% sugars, 3.22% lignin, and 3.34% acetic acid, and inhibitors in very less amount are produced when treated with dilute sulfuric acid [48, 49]. At 4 wt% concentration of sulfuric acid, pretreatment is preferred because of less cost and more effectiveness of the process. Dilute sulfuric acid causes biomass hydrolysis and then further breakdown of xylose into furfural is achieved. High temperature favors hydrolysis by dilute sulfuric acid [50]. Removal of hemicellulose is important to increase glucose yield from cellulose, and dilute sulfuric acid is very effective to achieve this purpose. It is necessary for an economical biomass conversion to achieve high xylan-to-xylose ratio. One-third of the total carbohydrate is xylan in most lignocellulosic materials. There are two types of dilute acid pretreatments, one is characterized by high temperature, continuous flow process for low solid loadings, and the other one is with low temperature, batch process and high solid loadings. Temperature and solid loadings for the first type are  $>160^{\circ}\text{C}$  and 5–10%, respectively, and for the second type, temperature and solid loadings are around  $<160^{\circ}\text{C}$  and 10–40%, respectively [51, 52].

Besides sulfuric acid and hydrochloric acid, other acids like oxalic acid and maleic acid are also used for the pretreatment of lignocellulosic biomass. Oxalic and maleic acids have high pKa value and solution pH as compared to sulfuric acid. Because of having two pKa values, dicarboxylic acids hydrolyze biomass more efficiently than sulfuric acid and hydrochloric acid. Other advantages include less toxicity to yeast, no odor, more range of pH and temperature for hydrolysis, and no hampering of glycolysis. Maleic acid has  $k_{\text{hyd}}/k_{\text{deg}}$ , due to which hydrolysis of cellulose to glucose is preferred over glucose breakdown. Effects of oxalic, sulfuric, and maleic acid pretreatment on biomass at the same combined severity factor (CSF) were determined [53]. The use of maleic acid produces high concentration of xylose and glucose as compared to oxalic acid.

### 3.2 Alkali pretreatment

Apart from acids, few bases are also used for pretreatment of biomass. Lignin contents greatly affected the result of alkaline treatment. As compared to other pretreatment methods, alkali treatment requires less pressure and temperature and ambient condition, but alkali pretreatment needs time in days and hours. Degradation of sugar in alkali treatment is less than that by acid treatment, and also the removal and recovery of caustic salt are possible and easy in case of alkali

treatment. Ammonium, sodium, calcium, and potassium hydroxides are used for alkaline pretreatment, but among these sodium hydroxide is the most commonly used alkaline pretreatment agent, whereas calcium hydroxide is the cheapest yet effective among all other alkali agents for pretreatment. By neutralizing calcium with carbon dioxide, calcium can be recovered easily in form of insoluble calcium carbonate. Using lime kiln technology, calcium hydroxide can be regenerated. Apparatus required for alkali pretreatment is basically temperature controller, a tank, CO<sub>2</sub> scrubber, water jacket, manifold for water and air, pump, tray, frame, temperature sensor, and heating element. The first step of pretreatment consists of making lime slurry with water. The next step is spraying of this slurried lime on biomass; after spray, store the biomass for hours or, in some case, days. Contact time can be reduced by increasing temperature [54–57]. Crystallinity index increases in lime pretreatment because of the removal of lignin and hemicellulose. Structural features resulting from lime pretreatment affect the hydrolysis of pretreated biomass. Correlation of three structural factors, viz., lignin, acetyl content and crystallinity, and enzymatic digestibility, was reported by Chang and Holtzapple [58]. He concluded that (1) regardless of crystallinity and acetyl content, in order to obtain high digestibility, extensive delignification is enough. (2) Parallel barriers to hydrolysis are removed by delignification and deacetylation. (3) Crystallinity does not affect ultimate sugar yield; however, it plays some role in initial hydrolysis. It is evident from these points that lignin content should be reduced to 10% and all acetyl groups should be removed by an effective pretreatment process. Thus in exposing cellulose to enzymes, alkaline pretreatment plays an important role. By increasing enzyme access to cellulose and hemicellulose and eliminating nonproductive adsorption sites, lignin removal can play its role in increasing effectiveness of enzyme.

### **3.3 Organosolv**

Aqueous organic solvents like methanol, acetone, ethanol, and ethylene glycol are used in this method with specific conditions of temperature and pressure. Organosolv pretreatment is usually performed in the presence of salt catalyst, acid, and base. The biomass type and catalyst involved decide the temperature of pretreatment, and it can go up to 200°C. Lignin is a valuable product, and to extract lignin this process is used mainly. Cellulose fibers are exposed when lignin is removed, which leads to more hydrolysis. During organosolv pretreatment, fractions and syrup of cellulose and hemicellulose, respectively, are also produced. There are certain variable factors like catalyst type, temperature, and concentration of solvent and reaction time which affects the characteristics of pretreated biomass like crystallinity, fiber length, and degree of polymerization. Inhibitor formation is triggered by long reaction, high temperature, and acid concentrations [59, 60]. In a study by Park et al. [61], effect of different catalyst was checked for the production of ethanol and among sulfuric acid, sodium hydroxide, and magnesium sulfate, and sulfuric acid was found to be most effective in ethanol production, but for enhancing digestibility the use of sodium hydroxide is proven to be effective. Sulfuric acid is a good catalyst, but its toxicity and inhibitory nature make it less favorite. Organosolv is not a cost-effective pretreatment process because of the high cost of catalysts, but it can be made cost-effective by recovering and recycling of solvents. Solvent removal is important because its presence affects fermentation, microorganism growth, and enzymatic hydrolysis. There is added risk of handling such harsh organic solvents. Acid helps in hydrolysis and depolymerization of lignin. Upon cooling lignin is dissolved in phenol, and in the aqueous phase, sugars are present. Formasolv involving formic acid, H<sub>2</sub>O, and hydrochloric acid is a type of organosolv



in which lignin is soluble and at low temperature process can be carried out. For pretreatment with ethanosolv cellulose, hemicellulose and pure lignin can be recovered, but high pressure and temperatures are required when ethanosolv is used, and less toxic nature of ethanol as compared to other organosolv makes it favorite for pretreatment. Ethanosolv when used in pretreatment effects the enzymatic hydrolysis, so to prevent this low ethanol, water is used [62]. Recovery of ethanol and water reduces the overall cost of the pretreatment. For sugarcane bagasse Mesa et al. [63] used ethanosolv at 195°C for 60 min, and results showed formation of 29.1% sugars from 30% ethanol. Alcohol-based organosolv pretreatment is combined with ball milling by Hiden et al. [24] to pretreat Japanese cypress and observed a synergistic effect on digestibility. 50.1, 41.7, and 48.1% yield of organosolv pulping was obtained from ethylene glycol-water, acetic acid-water, and ethanol-water in a study done by Ichwan and Son [64]. Poplar wood chips were first treated with steam and then with organosolv to separate cellulose, lignin, and hemicellulose. About 88% hydrolysis of cellulose to glucose, 98% recovery of cellulose, and 66% increase in lignin extraction were reported by Panagiotopoulos et al. [65].

### 3.4 Ionic liquids

For the pretreatment of lignocellulose, scientist took a great interest in using ionic liquids, for decades. Ionic liquids containing cations or anions are a new class of solvents with high thermal stability and polarity, less melting point, and negligible vapor pressure [66, 67]. Normally large organic cations and small inorganic anions compose ionic liquids. Factors like degree of anion charge delocalization and cation structure significantly effect physical, biological, and chemical ionic liquid properties. Interactions between ionic liquids and biomass get affected by temperature, cations and anions, and time of pretreatment.

Ionic liquids actually compete for hydrogen bonding with lignocellulosic components, and in this competition disruption of network occurs. 1-Ethyl-3-methylimidazolium diethyl phosphate-acetate, 1-butyl-3-methylimidazolium-acetate, cholinium amino acids, cholinium acetate, 1-ethyl-3-methylimidazolium diethyl phosphate-acetate, 1-allyl-3-methylimidazolium chloride, and chloride are ionic liquids used for the treatment of rice husk, water hyacinth, rice straw, kenaf powder, poplar wood, wheat straw, and pine. Among other ionic liquids are imidazolium salts which are most commonly used [42]. 1-Butyl-3-methylimidazolium chloride is used for pretreatment by Dadi et al. [68] who observed a twofold increase in yield and rate of hydrolysis. For the pretreatment of rice straw, Liu and Chen [69] used 1-butyl-3-methylimidazolium chloride also known as (Bmim-Cl) and observed significant enhancement in the process of hydrolysis due to modifications in the structure of wheat straw by Bmim-Cl. Bmim-Cl played role in the reduction of polymerization and crystallinity. A twofold increase in hydrolysis yield from sugarcane bagasse was observed in a study by Kuo and Lee [70] as compared to untreated bagasse. 1-Ethyl-3-methylimidazolium-acetate is used in a study by Li et al. [71] for the pretreatment of switch grass in order to remove lignin at a temperature of 160°C for 3 hours. Results showed 62.9% lignin removal enhanced enzymatic digestibility, and reduced cellulose crystallinity was reported by Tan et al. [72] on palm tree pretreatment with 1-butyl-3-methylimidazolium chloride. Slight changes in composition of biomass occurred after ionic liquid pretreatments although significant changes were observed in the structure of biomass. Ionic liquid pretreatment is less preferred over other techniques because of high thermal and chemical stability, less dangerous conditions for processing, low vapor pressure of solvents, and retaining liquid state at wide range of temperature. Ionic liquids can be recycled easily and are non-derivatizing. Disadvantage of using ionic liquid pretreatment is that



noncompatibility of cellulase and ionic liquids results in the unfolding and inactivation of cellulase. At less viscosity cellulose solubilizes at low temperature; that's why while using ionic liquids, viscosity is an important factor to be considered regarding the energy consumption of the whole process. High temperatures trigger more side reactions and negative side effects like reducing ionic liquid stability [73].

### **3.5 Ozonolysis**

Ozone pretreatment is a great option for lignin content reduction in lignocellulosic biomass. In vitro digestibility of biomass is enhanced by the application of ozone pretreatment. Inhibitors are not formed in this pretreatment which is a great advantage because other chemical pretreatments produce toxic residues. In ozone pretreatment, ozone acts as an oxidant in order to break down lignin. Ozone gas is soluble in water and being a powerful oxidant, by breaking down lignin, releases less molecular weight, soluble compounds. Wheat straw, bagasse, cotton straw, green hay, poplar sawdust, peanut, and pine can be pretreated with ozone in order to degrade lignin and hemicellulose; however, only slight changes occur in hemicellulose, whereas almost no changes occur in cellulose. Ozonolysis apparatus consists of ozone catalytic destroyer, iodine trap used for testing efficiency of catalyst, oxygen cylinder, ozone generator, three-way valve, ozone UV spectrophotometer, pressure regulation valve, process gas humidifier, vent, and automatic gas flow control valve [40, 41, 74–76]. Moisture content hugely effects oxidation of lignin via ozone pretreatment as lignin oxidation decreases with increase in the moisture content of biomass. Ozone mass transfer is limited at less water concentration, which ultimately effects its reactivity with biomass. Longer residence time of ozone is caused by the blockage of pores by water film [77]. During ozonolysis, pH of water decreases because of the formation of organic acids. Alkaline media trigger delignification because it removes lignins that are bonded to carbohydrates [78, 79].

Biomass delignification is associated with the production of inhibitory compounds. Certain aromatic and polyaromatic compounds are produced as a result of delignification [80]. Structural changes in lignin are observed by Bule et al. [81] in a study; different lignin subunits showed aromatic opening and degradation of  $\beta$ -O-4 moieties in NMR analysis. How do aromatic structures of control- and ozone-pretreated samples differ? A spectrum showed a decrease in aromatic carbon signal concentration. Changes were observed in methoxy groups that suggest the breakdown of ester-linked structure. Different reactor designs are used for the ozone pretreatment of biomass, for example, batch reactor, Drechsel trap reactor, fixed bed reactor, rotatory bed reactor, and multilayer fixed bed reactor. Plug flow reactors are used by most researchers [82]. Heiske et al. [83] compared the characteristics of single layered and multiple layered bed reactors in order to improve the wheat straw conversion to methane. Straw with 16.2% lignin concentration was obtained from single layered reactor, whereas in multiple layered reactor, lignin concentration decreased up to 7.2% at the bottom layer. Due to wax degradation in ozone-pretreated wheat straw, production of fatty acid compounds is observed by Kádár et al. [84]. About 49% lignin degradation was observed when corn stover was pretreated with ozonolysis in a study by Williams [85].

## **4. Physicochemical pretreatment**

### **4.1 Ammonia fiber expansion (AFEX)**

AFEX technique belongs to the category of physicochemical pretreatment methods. In this low temperature process, concentrated ammonia (0.3–2 kg ammonia/

kg of dry weight) is used as a catalyst. Ammonia is added to biomass in a reactor of high pressure; after 5–45 min of cooking, pressure is released rapidly. Normally temperature around 90°C is used in this process. Ammonia can be recovered and reused because of its volatility. The principle of AFEX is similar to steam explosion. Apparatus for AFEX includes reactor, thermocouple well, pressure gauge, pressure relief valve, needle valve, sample cylinder, temperature monitor, and vent. Rate of fermentation is seen to be improved by AFEX pretreatment of various grasses and herbaceous crops. For treatment of alfalfa, wheat chaff and wheat straw AFEX technology is used. Hemicellulose and lignin cannot be removed by using AFEX technology; hence, small amount of material is solubilized only. Degradation of hemicellulose into oligomeric sugars and deacetylation occur during AFEX pretreatment which is the reason of hemicellulose insolubility. After AFEX pretreatment of Bermuda grass and bagasse, 90% hydrolysis of cellulose and hemicellulose was achieved. Effectiveness of AFEX pretreatment decreases with increase in the lignin content of biomass, for example, newspaper, woods, nutshells, and aspen chips. In case of AFEX pretreatment for newspaper and aspen chips, maximum hydrolysis yield was 40% and 50%, respectively. So for the treatment of biomass with high lignin content, AFEX pretreatment is not a suitable choice.

Ammonia recycle percolation (ARP) is another method that uses ammonia. Aqueous ammonia (10–15 wt %) is used in this method. With a fluid velocity of 1 cm/min and temperature of 150–170°C and residence time of 14 minutes, aqueous ammonia passes through biomass in this pretreatment, and ammonia is recovered afterwards. Under these conditions, ammonia reacts with lignin and causes the breakdown of lignin breakdown linkages. Liquid ammonia is used in AFEX technique whereas in ammonia recycle percolation method/technique, aqueous ammonia is used.

## 4.2 Steam explosion

In this method, high-pressure saturated steam is used to treat lignocellulosic biomass, and then suddenly pressure is reduced, due to which lignocellulosic biomass undergoes explosive decompression. Initiation temperature of steam explosion 160–260°C and 0.69–4.83 MPa pressure is provided for few seconds to minutes, and then lignocellulosic biomass is exposed and retained at atmospheric pressure for a period of time; this triggers hydrolysis of hemicellulose and at the end explosive decompression, terminated the whole process. Cellulose hydrolysis potential increases due to the cellulose degradation and lignin transformation caused by high temperature. During the steam explosion pretreatment, acid and other acids formed, which played their role in the hydrolysis of hemicellulose. Fragmentation of lignocellulosic material occurs due to turbulent material flow and rapid flashing of material to atmospheric pressure [86–88]. In steam explosion pretreatment, the use of sulfuric acid or carbon dioxide decreases time, temperature, and formation of inhibitory products and increases hydrolysis efficiency that ultimately leads to complete removal of hemicellulose. Steam explosion pretreatment is not that effective for pretreating soft woods; however, acid catalyst addition during the process is a prerequisite to make the substrate accessible to hydrolytic enzymes. By using steam, targeted temperature can be achieved to process the biomass without the need of excessive dilution. Sudden release of pressure quenches the whole process at the end and also lowers the temperature. Particulate structure of biomass gets opened by rapid thermal expansion which is used to terminate the reaction. Steam explosion gets affected by certain factors like moisture content, residence time, chip size, and temperature. By two ways optimal hydrolysis and solubilization of hemicellulose can be achieved; either use high temperature and short residence

time or low temperature and high residence time. Low energy requirement is a great advantage of steam explosion pretreatment, whereas in mechanical pretreatment 70% more energy is required as compared to steam explosion pretreatment in order to obtain the same, reduced particle size. So far steam explosion pretreatment with addition of a catalyst is tested and came closest to scaling up at commercial level due to its cost-effectiveness. In Canada, at Iogen demonstration plant, steam explosion pretreatment is used at a pilot scale. For hardwood and agriculture residues, steam explosion pretreatment is a very effective pretreatment process.

### **4.3 Carbon dioxide explosion**

Supercritical carbon dioxide explosion treatment falls in the category of physiochemical pretreatment. Scientists had tried to develop a process cheaper than ammonia fiber explosion and a process which would operate at temperature lower than steam explosion temperature. In this process, supercritical carbon dioxide is used that behaves like a solvent. Supercritical fluids are compressed at room temperature above its critical point. When carbon dioxide is dissolved in water, carbonic acid is formed which causes less corrosiveness due to its special features. During the process, carbon dioxide molecules enter into small pores of lignocellulosic biomass due to its small size. Carbon dioxide pretreatment is operated at low temperature which helped in prevention of sugar decomposition by acid. Cellulosic structure is disrupted when carbon dioxide pressure is released which ultimately increased the accessibility of the substrate to the cellulolytic enzymes for the process of hydrolysis [11, 40, 41, 43]. Dale and Moreira [89] used carbon dioxide pretreatment for alfalfa and observed 75% theoretical release of glucose. Zheng et al. [90] performed experiments to show comparison among ammonia explosion, steam pretreatment, and carbon dioxide pretreatment of recycled paper and sugarcane bagasse. The results showed that carbon dioxide explosion pretreatment is cost-effective than AFEX.

### **4.4 Liquid hot water (LHW)**

Hot compressed water is another terminology used for this method of treatment. High temperature (160–220°C) and pressure (up to 5 MPa) are used in this type of pretreatment in order to maintain the liquid state of water. However, chemicals and catalysts are not used in liquid hot water pretreatment method [42]. In this method, water in liquid form remains in contact with lignocellulosic biomass for about 15 min. In this treatment pressure is used to prevent its evaporation, and sudden decompression or expansion in this pretreatment process is not needed. This method has proved to be very effective on sugarcane bagasse, wheat and rye straw, corncobs, and corn stover. Different terms like solvolysis, aqueous fractionation, aquasolv, and hydrothermolysis are used by different researchers to describe this pretreatment method [42, 60, 91]. Based on biomass flow direction and water flow direction into reactor, liquid hot water pretreatment can be performed in three different ways. The first method is co-current pretreatment, which is carried out by heating biomass slurry and water at high temperature, holding it for a controlled residence time at pretreatment conditions, and finally applying cool environment. The second method involves the countercurrent pretreatment that engages pumping of hot water against biomass at controlled conditions. The third method is the flow-through pretreatment, which can be carried out by the flow of hot water through lignocellulosic biomass which acts like a stationary bed.

To investigate the effect of liquid hot water pretreatment, a study was conducted by Abdullah et al. [92] that determined the different hydrolysis rates of cellulose



and hemicellulose. Two steps of optimization of various conditions were considered. The first step was performed at less severity for hydrolyzing hemicellulose, whereas the second step was performed at high severity for cellulose depolymerization. Disadvantage of liquid hot water pretreatment is high energy consumption requirement for downstream process because of the involvement of large amount of water. However, the advantage of this process is that chemicals and catalysts are not required and no inhibitor is formed [60].

#### 4.5 Wet oxidation

In this pretreatment method, oxygen/air and water or hydrogen peroxide is used to treat biomass at high temperatures ( $>120^{\circ}\text{C}$ ) for half an hour at 0.5–2 MPa pressure [11, 93]. This pretreatment method is also used for the treatment of waste water and soil remediation. This method has proven to be very effective for pretreatment of lignin enriched biomass. Certain factors like reaction time, oxygen pressure, and temperature effect the efficiency of wet oxidation pretreatment process. Water acts like acid at high temperature, so it induces hydrolysis reaction as hydrogen ion concentration increases with increase in temperature which ultimately decreases the pH value. Pentose monomers are formed as a result of hemicellulose breakdown in wet oxidation pretreatment, and oxidation of lignin occurs, but cellulose remains least affected. There are certain reports on the addition of alkaline peroxide or sodium carbonate. The addition of these chemical agents help in bringing down temperature reaction and reduce the formation of inhibitory compounds. Efforts to improve the degradation of hemicellulose at high temperature lead to the formation of inhibitory compounds like furfural and furfuraldehydes. However, amount of the production of inhibitors in wet oxidation pretreatment is certainly less than that of liquid hot water pretreatment or steam explosion method. There is extremely less possibility of using this process at industrial scale because of two reasons. One is the combustible nature of oxygen, and the other is the high cost of hydrogen peroxide used in the process [94].

#### 4.6 SPORL treatment

SPORL stands for sulfite pretreatment to overcome recalcitrance of lignocellulose, and this technique is used for pretreatment of lignocellulosic biomass [95]. SPORL is performed in two steps. The first step involves treatment of biomass with magnesium or calcium sulfite for the removal of lignin and hemicellulose fractions. The second step involves the reduction in size of pretreated biomass via mechanical disk miller. Effect of SPORL pretreatment was studied by Zhu et al. [22, 23] on spruce chips by employing conditions like temperature  $180^{\circ}\text{C}$ , half an hour time duration, 8–10% bisulfite, and 1.8–3.7% sulfuric acid. By employing these conditions, more than 90% substrate was converted to cellulose when cellulase of 14.6 FPU and 22.5 CBU  $\beta$ -glucosidase was used in hydrolysis. Low-yield inhibitors like hydroxymethyl furfural (HMF) (0.5%) and furfural (0.1%) were produced during this process. These percentages are far less as compared to acid-catalyzed steam pretreatment of spruce. In another study, SPORL-pretreated Poplar NE222, beetle-killed lodgepole pine, and Douglas fir were purified. Low contents of sulfur and molecular mass were obtained with high phenolic derivative production [96].

SPORL pretreatment on switch grass with temperature ranging between  $163$  and  $197^{\circ}\text{C}$ , 3–37 min time duration, 0.8–4.2% sulfuric acid dose, and 0.6–7.4% sodium sulfite dose was performed by Zhang et al. [97]. The results with enhanced digestibility by the removal of hemicellulose due to sulfonation and decreased hydrophobicity of lignin were obtained. SPORL yielded 77.3% substrate as compared to 68.1%



for dilute acid treatment and 66.6% through alkali pretreatment. When sodium sulfite, sodium hydroxide, and sodium sulfide were used in SPORL pretreatment of switch grass, an improved digestibility of switch grass was achieved. When SPORL treatment was applied with optimized conditions, 97% lignin and 93% hemicellulose were removed from water hyacinth, and 90% hemicellulose and 75% lignin were achieved for rice husk [98].

## 5. Biological pretreatment

Conventional methods for chemical and physical pretreatments require expensive reagents, equipment, and high energy. On the other hand, biological pretreatment requires live microorganisms for the treatment of lignocellulosic material, and this method is more environment friendly and consumes less energy. There are certain microorganism present in nature that exhibit cellulolytic and hemicellulolytic abilities. White-rot, soft-rot, and brown fungi are known for lignin and hemicellulose removal with a very little effect on cellulose. White rot is able to degrade lignin due to the presence of lignin degrading enzymes like peroxidases and laccases. Carbon and nitrogen sources are involved in the regulation of these degrading enzymes [41]. Cellulose is commonly attacked by brown rot, whereas white and soft rot target both lignin and cellulose contents of plant biomass. Commonly used white-rot fungi species are *Pleurotus ostreatus*, *Ceriporiopsis subvermispora*, *Ceriporia lacerata*, *Pycnoporus cinnabarinus*, *Cyathus cinnabarinus*, and *Phanerochaete chrysosporium*. Basidiomycetes species including *Bjerkandera adusta*, *Ganoderma resinaceum*, *Trametes versicolor*, *Fomes fomentarius*, *Irpex lacteus*, *Lepista nuda*, and *Phanerochaete chrysosporium* are also tested, and these species showed high efficiency for delignification [41, 99].

Pretreatment of wheat straw was studied by Hatakka [100]. The results showed 13% conversion of wheat straw into sugars by *Pleurotus ostreatus* in duration of 5 weeks, whereas *Phanerochaete sordida* and *Pycnoporus cinnabarinus* showed almost the same conversion rate but in less time. For degradation of lignin in woodchips and to prevent cellulose loss, cellulase-less mutant of fungus *Sporotrichum pulverulentum* was developed [101]. Delignification of Bermuda grass by white-rot fungi *Ceriporiopsis subvermispora* and *Cyathus stercoreus* was studied that resulted in 29–32 and 63–77% improvement in delignification [102]. During the secondary metabolism in fungus *P. chrysosporium*, two lignin degrading enzymes, lignin peroxidase and manganese-dependent peroxidase, are produced in response to carbon and nitrogen limitation. Extracellular filtrates of various white-rot fungi contain these two enzymes.

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## Author details

Muhammad Nauman Aftab<sup>1\*</sup>, Irfana Iqbal<sup>2</sup>, Fatima Riaz<sup>1</sup>, Ahmet Karadag<sup>3</sup>  
and Meisam Tabatabaei<sup>4</sup>

<sup>1</sup> Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan

<sup>2</sup> Department of Zoology, Lahore College for Women University, Lahore, Pakistan

<sup>3</sup> Faculty of Science, Bartın University, Bartın, Turkey

<sup>4</sup> Agricultural Biotechnology Research Institute of Iran (ABRII), AREEO, Karaj, Iran

\*Address all correspondence to: [nauman535@yahoo.com](mailto:nauman535@yahoo.com)

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