A Feasibility Study about Cellulosic Ethanol Industrialization

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Abstract. The second-generation of biomass ethanol, also known as cellulosic ethanol, is a major topic of discussion. The immaturity of pretreatment technology is one of the main factors restricting the scale-up of the second-generation bio-ethanol technology. Appropriate pretreatment methods can improve the accessibility of cellulose, reducing the cost of separating cellulose, hemicellulose and lignin, while increasing the yield of cellulosic ethanol. Pretreatment methods are mainly divided into four categories, namely chemical pretreatment, physical and chemical pretreatment, physical pretreatment as well as biological pretreatment methods. By analyzing the principles, effects, advantages and disadvantages of the popular pretreatment methods, whether they were suitable for industrialization were assessed. According to the results, chemical treatment methods are most frequently used but restricted to the high cost, difficulty in recovery, the impact of organic solvents and the safety problems. Physicochemical pretreatment methods are popular but has the problem of high energy cost. Besides, biological methods are not suitable for industrialization due to the high time-consuming and low degradation efficiency. Physical pretreatment is currently only a supplement of chemical pretreatment and physicochemical pretreatment, while due to its advantages such as the efficiency of pretreatment, reduction of the dosage of chemicals and low cost, it is worth being studied in the future.

1. Introduction
In recent years, due to the fierce environmental problems causing by GHG, the voice of using biofuel instead of gasoline as energy source has become rising. Ethanol, a typical biomass outcome, can be used not only as fuel, but also as raw material of chemical products. In this case, producing bio-ethanol efficiently has been a popular subject because of its high application value. The technology of first-generation biomass-ethanol, produce ethanol through food crops, has been relatively mature. However, this technology is controversial, because the raw material of this technology is crops. Considering that there are still a large number of individuals suffering famine in the world, governments in developed countries take measures to avoid using food as raw material to produce fuel. Thus, the second-generation biomass ethanol technology emerged.

The second-generation bio-ethanol, which is also known as cellulosic ethanol, uses agricultural wastes such as straw and corncob as raw materials. The carbohydrate components are separated from agricultural wasted and being saccharified (hydrolysis) and fermented to produce ethanol. Since agricultural wastes have a wide range of sources as well as a low price, its biomass conversion has been defined as an important part of sustainable development society in the past decade.
Nevertheless, the main source of bio-ethanol is still the first generation of ethanol. The reason why the second-generation cellulosic ethanol cannot fully replace the previous one is that cellulosic ethanol has no cost advantage over the crops’ ethanol. Although it has the advantages of raw material cost and energy cost, this advantage is not reflected due to the high cost of enzyme used in hydrolysis of cellulose. An appropriate pretreatment method is capable to inverse this situation through increasing the accessibility of cellulose by destroying the crystal structure of lignocellulose. In a word, pretreatment methods can enhance enzyme function and reducing its consumption which bring cost advantage back to the cellulosic ethanol.

Large amounts of laboratory experiments about pretreatment methods are done recently. Unfortunately, due to the difficulty to analyze the economic benefits of the new pretreatment methods in industrialization, the most basic pretreatment methods are still widely used at present. The following will list the characteristics of various methods, compare their advantages and disadvantages, and provide analysis basis for the feasibility analysis of industrialized production.

2. Pretreatment requirement
The requirement for pretreatment on enzyme mainly includes the following three aspects.

2.1. Enhancing the accessibility of cellulose
Enhancing the accessibility of cellulose through removing lignin and hemicellulose is on top priority. Lignocellulose is mainly composed of 40% to 50% cellulose, 25% to 30% hemicellulose and 15% to 20% lignin. [1, 2] Due to the sleeve structure of lignocellulose, as shown in Figure 1, the primary wall containing a large amount of hemicellulose and lignin surrounds the secondary wall, resulting in a high degree of crystallinity, which makes the cellulose hydrolase unable to contact the internal secondary wall. Therefore, it is necessary to break down the crystal of lignocellulose through appropriate methods to increase the accessibility of cellulose and improve the treatment efficiency of cellulase.

![Figure 1. Plant cell wall structure model.](image)

2.2. Minimize the cellulose hydrolysis
The second aspect is to minimize the cellulose hydrolysis (peeling reaction) during pretreatment. If the reaction conditions are too extreme, for example, the temperature is too high or the concentration of acid or alkali is too high, the field of ethanol will drop. At temperatures above 100°C, an average of nearly 50 glucose molecules were removed before termination [3]. At higher temperatures (>170°C), hydrolysis can occur at any site of cellulose and the yield will be even lower [4]. Thus, though some pretreatment methods can break down the crystal of lignocellulose in an extreme condition, we need to
avoid using them. Using anthraquinone (AQ) in pretreatment is profitable. It can not only eliminate the peeling reaction, but also attack lignin as a nucleophilic reagent, which is helpful for delignification.

2.3. Reducing by-products harmful to enzymes
An appropriate pretreatment not only conducive to the subsequent enzymatic hydrolysis of lignocellulose, reduce the crystallinity of lignocellulose, but also can cut down the formation of substances that reduce enzyme activity and improve the working conditions of enzyme, thus minimize the cost by reducing enzyme usage.

Recently, some researchers find that reducing the by-products of pretreatment, such as furans, organic acids and phenolics, have a large impact on ethanol productivity. At the ratio of 150 mg phenol / mg protein (enzyme), the final glucose production was reduced by 80% compared with the normal condition [5]. The inhibitory by-product mainly include furfural and 5-hydroxymethyl furfural (HMF), which are dehydration products of pentose and hexose respectively. (figure. 2) It should be noted that furfural is easy to decarboxylate and produce furan when the temperature is 200°C-220°C. Therefore, the operating temperature of pretreatment methods should be away from this range as far as possible.

Figure 2. Hemicellulose hydrolysate.

Though limiting pretreatment circumstance can minimizing by-product which will benefit the subsequent enzymatic hydrolysis step, it reduces the efficiency of pretreatment. Another solution can also be chosen, to detoxify the raw materials after pretreatment, including chemical methods, physical methods and biological methods. Recent advances are shown in the Table1.

| Type                | Name              | Advantage               | Drawback                                                      |
|---------------------|-------------------|-------------------------|----------------------------------------------------------------|
| Chemical Method     | Reductive detoxification | Easy to prepare Environment protection | Low efficiency                                                 |
|                     | Base              | High efficiency         | Great quantity demand; Hard to recover; Low selectivity and efficiency for detoxification |
|                     | Amino acid        | High efficiency         | Change Fermentation Path and Product [6]                      |
In recent years, compound detoxification method has become a hot research direction [8-11]. The improvement effect of deoxidation on the subsequent fermentation process and whether it can be recycled and reused (whether the cost is reasonable) should be considered comprehensively through the actual production of raw material varieties and production conditions. Therefore, the cost coupling calculation between pretreatment and detoxification can be considered to find out the optimal scheme.

3. Pretreatment Technologies:

3.1. Chemical Pretreatment Technologies

The chemical pretreatment methods mainly use chemical reagents to remove part of lignin or hemicellulose by hydrolysis and dissolution, which make the overall crystallinity decrease and the inner layer exposed.

3.1.1. Dilute acid pretreatment and alkaline pretreatment. Due to the early start and long development time of acid and alkali pretreatment, many large-scale cellulosic ethanol plants take it as the main means of pretreatment. For example, Abengoa bioenergy company built a biomass ethanol plant in Kansas State in the United States and the biomass ethanol plant with an annual output of 54000 T/year built by Logan biorefining company in Canada, both adopt dilute acid as the main pretreatment method. Acid pretreatment mainly uses the relative instability of hemicellulose glycoside bond to hydrolyze hemicellulose; alkali pretreatment mainly aims at lignin. Dilute acid or hydrothermal pretreatment is able to remove a huge amount of hemicellulose from a solid matrix and obtain a xylo-oligomer-rich liquid fraction. [12] However, acid pretreatment will cause environmental pollution, corrode equipment, toxic waste water, and waste a lot of chemicals and generate solid waste in the neutralization process.

3.1.2. Ionic liquid pretreatment. In recent years, ionic liquid (IL) has become a hot research object of biomass pretreatment. Because of their large size, asymmetry and conformational flexibility, the melting point of ionic liquids is usually below 100°C. [13, 14] Cation and anion groups can be designed to confer specific properties, such as low vapor pressure, specific solubility of solutes, or high miscibility with other solvents [14-17]. In 2002, Swatloski et al. [18] first found that imidazole ionic liquids can dissolve cellulose. The dissolution mechanism of cellulose in ionic liquids is mainly based on the electron donor acceptor (EDA) theory. The cation of the liquid acts as the electron acceptor, and the anion acts as the electron donor. The hydrogen bond between cellulose macromolecules is broken through the interaction between anion and cation of ionic liquid and -OH in cellulose, so as to realize the dissolution of cellulose. Like many new pretreatment technologies, ionic liquids have many advantages, but due to the cost of synthesis and recovery, it has not been able to be used on a large scale. Recently, Ferrari suggests that [Mea]+-based IL is a cheaper and more effective alternatives to [Emim][Ac] and cholinium-based IL [19].
3.1.3. Deep eutectic solvent pretreatment. Deep eutectic solvent (DES) is a kind of eutectic mixture formed by the force between the hydrogen bond donor and the hydrogen bond receptor. Its physical and chemical properties are similar to that of IL [20]. IL is pure substance, while DES is a mixture which is molten salt (composed of anions and cations) at room temperature. Because of its easiness in synthesis, stability and low cost, DES is generally considered to be superior to IL in preparation and application. It is reported that the cost of DES is only 20% of that of IL [21]. Gorke [22] showed that the cost of DES is only 1/10 of that of IL, and its constituent materials are almost from environmentally friendly substances. Hemicellulose is much more difficult to remove by DES. Under strong acid conditions, DES needs 120°C for about 4 hours to remove hemicellulose, while only a small amount of hemicellulose is removed in a moderate acid condition.

DES has the ability to supply and receive protons, dissolve lignin and xylan, and promote the dissociation of the three components [23]. DES has great solubility for lignin, especially acidic DES such as lactic acid, malic acid and oxalic acid [24]. Results illustrate that through DES pretreatment, lignin could be removed in many kinds of feedstock and the removal rate was up to 90% [25, 26]. It can specifically remove lignin and maintain the carbohydrate. Furthermore, DES can provide a mild double acid-base catalytic mechanism to promote the controlled cleavage of unstable ether bonds between phenylpropanoid units, so as to separate lignin from cellulose and depolymerize lignin, and produce low molecular weight lignin products, but it does not affect the C-C bond in lignin, and basically maintains the structural characteristics of lignin (β-β, β-5 bond, etc.) It is more conducive to the high value utilization of lignin in the following steps.

In addition, DES has good biocompatibility, that is, it has good compatibility with nucleic acids, proteins, enzymes and microorganisms. Moreover, DES has a wide range of applications in bio organic catalysis and transformation, and molecules [27]. DES can dissolve and extract high quality lignin with purity >90%, as well as 60% lignin from rice straw [28, 29]. Cellulose is almost insoluble in DES [24].

However, considering that the DES contain organic solvent using in extraction this method. Organic solvent can only be separated by distillation, which is a high energy consumption process. Higher reaction temperature also increases risk and cost of pretreatment process. What's more, if a small part of organic solvent remains in raw material, the efficiency of enzyme treatment will be greatly reduced in the process of subsequent enzyme treatment.

3.1.4. Advanced organic solvent pretreatment. For a long time, organic solvents have been criticized for their high requirements of reaction conditions and environmental damage. γ-valerolactone(GVL), as a green organic solvent derived from biomass, can solve this problem. Li [30] found that the pretreatment of hardwood with GVL and water (8:2) solvent system at 120°C and 75 mmol/L H₂SO₄ loading can remove up to 80% of the original lignin, while 96%-99% of the original cellulose is retained in the pretreated substrate; the pretreated substrate is quantitatively converted into The total yields of monosaccharide, glucose and xylose were 99% and 100%. These conversions are three times higher than those with other organic solvents (such as tetrahydrofuran or ethanol) and 20 times higher than those with pure water. In addition to high sugar yield, GVL pretreatment has great advantages in solvent
recovery and cost reduction. More than 99.5% of GVL can be recovered by \( \text{CO}_2 \) extraction of pretreatment pulp [31].

In recent studies, Wang proposed a biomass pretreatment method using sulfolane / water mixture under sulfuric acid conditions [32]. Sulfolane is a green organic solvent with excellent stability and water solubility. It has attracted wide attention due to its potential application in biorefinery. [32,33] Zhou demonstrated that the structure of lignin pretreated by sulfolane under sodium hydroxide condition was well preserved and could realize high value utilization. [34]

3.2. Physical Pretreatment Technologies

Physical method is usually used as an auxiliary means to mechanically break down the outer barrier of lignocellulose and break lignocellulose into smaller part. These methods result in increasing the accessibility of cellulose and increasing the efficiency of chemical pretreatment, making the overall pretreatment more efficient.

3.2.1. Ultrasonic pretreatment. Ultrasonic pretreatment is to crush the cellulose primary wall and other outer layers in the complex mechanism of ultrasonic fragmentation of polymer. Ultrasonic vibration produces cavitation effect, which promotes the fragmentation of lignocellulose polymer structure, thus increasing the accessibility of cellulose. Ma [35] believes that ultrasonic pretreatment destroys the ester bond between lignin. It is also found that ultrasonic assisted treatment is helpful to remove and destroy the wax layer and silica body deposited on the surface of lignocellulose structure, so as to reduce the particle size of lignocellulose [36].

3.2.2. Microwave radiation pretreatment. Microwave radiation, by virtue of the influence of microwave on molecular motion, micro performance is to promote friction and collision between molecules, destroy the crystal structure of lignocellulose, so as to reduce the crystallinity of lignocellulose, increase the accessibility of cellulose, and meet the requirements of homogeneity of material conversion technology [37, 38]. In the microwave-assisted pretreatment of biomass, lignin can be removed efficiently, and the chemical reactivity and processability of biomass cellulose can be effectively improved [39].

This method is easy to operate, environment-friendly, high energy efficiency, and also is able to change the ultrastructure of lignocellulose. As an auxiliary means of DES pretreatment, it can be carried out in low energy input, short time, simple reactor configuration and unit operation, which helps to reduce the pretreatment cost. Under the same treatment level, the reaction time can be shortened, which is conducive to scale-up and industrialization.

3.3. Physicochemical Pretreatment Technologies

3.3.1. Hydrothermal pretreatment. Hydrothermal pretreatment is one of the most potential pretreatment methods for large-scale industrialization utilize. Extra reagent is not used in the method. Under high temperature conditions (160–240°C), water autoionization produces oxygen ions to form hydrated ions. The glycosidic bond in hemicellulose is particularly impressed by the hydrated hydrogen ion, which makes hemicellulose depolymerized and dissolved from the biomass matrix, so as to eliminate the space hindrance to cellulase and improve the enzymatic hydrolysis efficiency [40]. The components of corn cob extract after hydrothermal pretreatment were mainly composed of glucose, xylobiose, xylotriose and xylotetraose [41]. Compared with other pretreatment methods, hydrothermal pretreatment method does not need to add any reagents, and the hydrolysis process rarely produces by-products that can inhibit the effect of cellulase [42]. As a new green environmental protection treatment technology, hydrothermal pretreatment has become a research hotspot in recent years. However, high reaction conditions of hot water pretreatment can make cellulose lose, and hemicellulose hydrolysis produces different types of short carbon chain sugars, and the requirements of enzyme are also improved.
3.3.2. Steam explosion pretreatment. Steam explosion pretreatment is another popular physical and chemical pretreatment technology. The mechanism of steam explosion pretreatment includes three aspects. The first aspect is similar to the hydrothermal pretreatment. Under the condition of high temperature and high pressure, the structure of lignocellulose is softened and part of hemicellulose is hydrolyzed. The second aspect is to destroy the hydrogen bond structure of lignocellulose. Steam, which have high temperature and pressure, permeate into the pores of straw fiber and form hydrogen bonds on the hydroxyl groups of cellulose molecular chain. At the same time, under the condition of high temperature, high pressure and water content, the hydrogen bond in cellulose will be destroyed and new hydroxyl groups will be released. When the high-pressure steam is released instantaneously, the hydrogen bond breaks, and the cellulose environment will rapidly cool to room temperature, so that the cellulose supramolecular structure is retained, increasing the adsorption capacity of cellulose, and further causing the destruction of residual hydrogen bonds, accelerating the movement of glucose ring group, and finally leading to the destruction of other crystal regions. The third aspect is the structural rearrangement. After the hydrogen bond of straw cellulose is destroyed, the mobility of cellulose chain is increased, which makes the cellulose chain rearrange, which is helpful to change to ordered structure [43, 44]. The steam explosion device is mainly composed of steam boiler, reactor and explosion tank. The steam boiler provides high-temperature and high-pressure steam for the reactor. After reaching a certain pressure and maintaining for a certain period of time, the sudden pressure drop is controlled by the blasting valve. During the decompression process, straw raw materials are discharged from the reactor and recycled in the explosion tank. In addition, the stirring or cleaning device can reduce the residual lignin on the surface, which can effectively improve the separate efficiency of lignin.

3.3.3. Ammonia fiber expansion/explosion. Besides, Ammonia fiber expansion/explosion (AFEX) is a popular pretreatment method known as abundant advantages. The principle is similar to that of hydrothermal pretreatment. Due to the good biocompatibility of ammonia, AFEX can enter into lignocellulose more effectively, effectively destroy the submicroscopic structure of cell wall, cut off the connection between lignin and polysaccharide, partially depolymerize hemicellulose and lignin, produce crystallization effect on fibroin, remove acetyl group on hemicellulose side chain, and improve cellulose enzyme Accessibility and enzymatic hydrolysis efficiency [45, 46]. In addition, AFEX pretreatment process also has the advantages of high solid loading rate, no need of water washing, the vast majority of ammonia can be recovered, and the residual ammonia can provide nitrogen source for the fermentation process [47, 48].

3.4. Biological Pretreatment Technologies
The main biological pretreatment method is using white rot fungus to digest lignin. The principle of biological pretreatment is to use enzymes produced by microorganisms, mainly catalase and laccase, to degrade lignin, remove its wrapping effect on cellulose, and improve the accessibility of cellulose. The most commonly used biological and treatment mold is white rot fungus. In the process of culture, the fungus can produce the transferase which can decompose lignin, and reduce the production of by-products which can inhibit the fermentation enzyme.

However, due to the low conversion rate, long reaction time and relatively low efficiency, the conversion rate of white rot fungus strain for lignin is 60% [49], so it is difficult to be applied to actual industrial production. Therefore, the best use scenario of biological pretreatment is in the storage period of raw materials. After farmers have collected straw and corncob, they should first put them into the warehouse suitable for the growth of white rot fungi for stacking. Through this method, we can decompose part of lignin in advance, reduce part of transportation cost, reduce the dosage of follow-up chemicals and reduce the cost of chemicals. However, this method requires agricultural intensification, and the management cost of scattered agricultural areas will be relatively high, so the implementation of this idea is very difficult.
4. Conclusion
The most frequently used pretreatment methods are chemical treatment methods, such as organic solvent pretreatment and ionic liquid pretreatment. However, due to the high cost of chemical synthesis, difficulty in recovery, the impact of organic solvents on enzyme activity and the hidden danger of factory safety, their large-scale use will be greatly restricted. Besides, pretreatment methods need to make a trade-off between profitable by-product recovery and reagent recovery in the subsequent separation steps, comprehensively comparing the recovery cost and profit (reagent synthesis cost and reduced wastewater treatment cost) of recovered reagent with the by-product extraction cost and profit brought by high-value processing. Assuming that the recovery cost is close, the synthetic price of reagents with excellent pretreatment capacity is generally higher. The high value processing of by-products needs to extend the industrial chain, with high input costs, and the limited profit of cellulose ethanol, which even needs to rely on government subsidies to the maintenance of the plain when the price of gasoline is low. Generally speaking, chemical pretreatment methods based on the cost of recovery reagent will be more likely be utilized in actual production.

Physicochemical pretreatment methods, such as steam explosion, are popular because they are reagent free process. Factory designer don’t need to focus on reagent recovery. The efficiency of separation is sublime. However, the main concern to the method is its high energy cost because remaining heat resource cannot be captured and reused.

Physical pretreatment, as a supplement of chemical pretreatment and physicochemical pretreatment, can improve the efficiency of pretreatment, reduce the dosage of chemicals and reduce the cost of treatment. In the future, the research on physical pretreatment will mainly focus on reducing energy consumption and focusing on the adaptability of existing processes. Microwave radiation, because of its advantages of easy to operate, environment-friendly, high energy efficiency and so on, is one of the pretreatment methods worth studying.

At present, biological method is the least commonly used method, because it is time-consuming and low degradation efficiency, it is not suitable for industrialization. But the biological method will also play an useful role in the future. The harvest time of agricultural products is usually a fixed time, but in order to meet the continuous production of the factory, it is necessary to store the raw materials in the warehouse, and white rot fungus can be used for pretreatment in the storage process. In addition, white rot fungus can also be used as a means of wastewater treatment.

All in all, the pretreatment methods need to be combined with complementary advantages. For example, reasonable use of physical method to reduce the total agent consumption. Using steam explosion are able to improve the removal rate of hemicellulose. The calculation of how long will it take to earn back the investment cost for an extra process’ equipment fee and maintenance cost will illustrate whether the combination of pretreatment methods is reasonable or not.

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