



INTERNATIONAL JOURNAL OF PURE AND APPLIED RESEARCH IN ENGINEERING AND TECHNOLOGY

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COMPREHENSIVE STUDY OF PRETREATMENT METHODOLOGIES FOR THE HYDROLYSIS OF LIGNOCELLULOSIC MATERIALS: A CRITICAL REVIEW

BHETALU A.D.¹, PATIL S. S.², GUPTA M. M.³

1. Assistant Professor, Department of Engineering Chemistry, IBSS College of Engineering, Amravati, Maharashtra, India.
2. Director, Student Welfare, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India.
3. Assistant Professor, Department of Engineering Chemistry, IBSS College of Engineering, Amravati, Maharashtra, India.

Accepted Date: 27/02/2014 ; Published Date: 01/05/2014

Abstract: Energy has a crucial role to play in the economic development of India. Much attention has been given for finding the alternative sources to crude oil due to its limited availability. New sustainable sources of energy will be the key in the future. Lignocellulosic biomass can be utilized to produce ethanol, a promising alternative energy source crude oil. The conversion of this biomass to ethanol can be achieved via hydrolysis of cellulose in the lignocellulosic biomass to produce reducing sugars, and subsequent fermentation of the sugars to ethanol. Various pretreatment methods can be employed to enhance the hydrolysis of cellulose. Optimization of the ethanol yield after fermentation is the main focus of hydrolysis.

Keywords: Ethanol; Fermentation; Hydrolysis; Lignocellulosic biomass; Pretreatment

Corresponding Author: MR. BHETALU A. D.



PAPER-QR CODE

Access Online On:

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How to Cite This Article:

AD Bhetalu, IJPRET, 2014; Volume 2 (9): 100-110

INTRODUCTION

Over 1.5 trillion barrels of oil equivalent have been produced since Edwin Drake drilled the world's first oil well in 1859. The world will need that same amount to meet demand in the next 25 years alone [1]. The consequences of inadequate oil availability could be severe. Therefore, it is imperative to explore alternative energy sources. Ethanol is a renewable energy source produced through fermentation of sugars. Ethanol is widely used worldwide as a partial gasoline replacement. The Government of India approved the "National Policy of Biofuels" on December 24, 2009. Since then, considerable advancements have taken place in the direction of cultivation, production and use of biofuels. The policy and the developments following it have strengthened India's energy security by encouraging the use of renewable energy resources to supplement transport fuels. It has stimulated rural development and has also created employment opportunities. It has addressed the global concern about containment of carbon emission through use of environmentally friendly biofuels.

Ethanol currently produced in India by the fermentation of sugarcane molasses. Due to limited agricultural land under cultivation of sugarcane also limits the production of ethanol from sugarcane. Presently, India accounts for 1% of fuel ethanol production. A potential source for low-cost ethanol production is to utilize lignocellulosic biomass (LCB) such as crop residues, grasses, sawdust, wood chips, and solid animal waste. Significant research has been done in this area since last 30 years. [2-8]

Lignocellulosic materials consist mainly of three polymers: cellulose, hemicellulose and lignin. These polymers are present in varying relative composition depending on the type, species and even source of the biomass [10]. The conversion of lignocellulosic material to ethanol is attributed to hydrolysis of cellulose to fermentable sugars and fermentation of these sugars to ethanol. The particular inherent structure of LCB is the main limiting factor of its conversion to ethanol. [11] Several factors affect the hydrolysis of cellulose to sugars viz. accessible surface area of the crystallinity, and lignin and hemicellulose content. The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis. Removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increase of porosity in pretreatment processes can significantly improve the hydrolysis [9].

Pretreatment is a crucial process step for the biochemical conversion of lignocellulosic biomass into bioethanol. It is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars [12]

2. Pretreatment of lignocellulosic materials:

The effect of pretreatment of lignocellulosic materials has been recognized for a long time. The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials.

Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (4) be cost-effective. [9]

There are several methods which have been introduced for the pretreatment of lignocellulosic material. These are classified into Physical pretreatment, physico-chemical pretreatments, chemical pretreatments and biological pretreatments [13]

2.1. Physical pretreatment:

2.1.1. Mechanical comminution:

Waste materials can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. The size of the materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding. Vibratory ball milling has been found to be more effective in breaking down the cellulose crystallinity of spruce and aspen chips and improving the digestibility of the biomass than ordinary ball milling [14]. The power requirement of mechanical comminution of agricultural materials depends on the final particle size and the waste biomass characteristics [4]

2.1.2. Pyrolysis

Pyrolysis has also been used for pretreatment of lignocellulosic materials. When the materials are treated at temperatures greater than 300 °C, cellulose rapidly decomposes to produce gaseous products and residual char [15],[16]

2.2. Physico-chemical pretreatment

2.2.1. Steam explosion (autohydrolysis):

Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials [9]. In this method, chipped biomass is treated with high-pressure saturated steam and then the pressure is swiftly reduced, which makes the materials undergo an explosive

decompression. Steam explosion is typically initiated at a temperature of 160–260 °C for several seconds to a few minutes before the material is exposed to atmospheric pressure. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis. Ninety percent efficiency of enzymatic hydrolysis has been achieved in 24 h for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips [17]. The factors that affect steam explosion pretreatment are residence time, temperature, chip size and moisture content [6]. Optimal hemicellulose solubilization and hydrolysis can be achieved by either high temperature and short residence time (270 °C, 1 min) or lower temperature and longer residence time (190 °C, 10 min). Recent studies indicate that lower temperature and longer residence time are more favorable [18].

Addition of H₂SO₄ (or SO₂) or CO₂ in steam explosion can effectively improve enzymatic hydrolysis, decrease the production of inhibitory compounds, and lead to more complete removal of hemicellulose [19]. The conventional mechanical methods require 70% more energy than steam explosion to achieve the same size reduction [20].

Steam explosion is recognized as one of the most cost effective pretreatment processes for hardwoods and agricultural residues, but it is less effective for softwoods [21]. Limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete disruption of the lignin–carbohydrate matrix, and generation of compounds that may be inhibitory to microorganisms used in downstream processes [21]. Because of the formation of degradation products that are inhibitory to microbial growth, enzymatic hydrolysis, and fermentation, pretreated biomass needs to be washed by water to remove the inhibitory materials along with water-soluble hemicellulose [9].

2.2.2. Ammonia fiber explosion (AFEX)

AFEX is another type of physico-chemical pretreatment in which lignocellulosic materials are exposed to liquid ammonia at high temperature and pressure for a Energy requirement of mechanical comminution of agricultural lignocellulosic materials with different size reduction [4]

In a typical AFEX process, the dosage of liquid ammonia is 1–2 kg ammonia/kg dry biomass, temperature 90 °C, and residence time 30 min. AFEX pretreatment can significantly improve the saccharification rates of various herbaceous crops and grasses. It can be used for the pretreatment of many lignocellulosic materials. The AFEX pretreatment does not significantly solubilize hemicellulose compared to acid pretreatment [22]. Over 90% hydrolysis of cellulose

and hemicellulose has been obtained after AFEX pretreatment of Bermuda grass (approximately 5% lignin) and bagasse (15% lignin) [23]. However, the AFEX process was not very effective for the biomass with high lignin content such as newspaper (18–30% lignin) and aspen chips (25% lignin).

To reduce the cost and protect the environment, ammonia must be recycled after the pretreatment. In an ammonia recovery process, a superheated ammonia vapor with a temperature up to 200 °C was used to vaporize and strip the residual ammonia in the pretreated biomass and the evaporated ammonia was then withdrawn from the system by a pressure controller for recovery [24]. The ammonia pretreatment does not produce inhibitors for the downstream biological processes, so water wash is not necessary [22],[25].

AFEX pretreatment does not require small particle size for efficacy [26].

2.2.3. CO₂ explosion

Similar to steam and ammonia explosion pretreatment, CO₂ explosion is also used for pretreatment of lignocellulosic materials. It was hypothesized that CO₂ would form carbonic acid and increase the hydrolysis rate. [27]. CO₂ explosion was more cost-effective than ammonia explosion and did not cause the formation of inhibitory compounds that could occur in steam explosion. [28]

2.3. Chemical pretreatment

2.3.1. Ozonolysis:

Ozone can be used to degrade lignin and hemicellulose in many lignocellulosic materials [29-32]. The degradation was essentially limited to lignin and hemicellulose was slightly attacked, but cellulose was hardly affected. Enzymatic hydrolysis yield increased from 0% to 57% as the percentage of lignin decreased from 29% to 8% after ozonolysis pretreatment of poplar sawdust [32]. Ozonolysis pretreatment has the following advantages: (1) it effectively removes lignin; (2) it does not produce toxic residues for the downstream processes; and (3) the reactions are carried out at room temperature and pressure [32]. However, a large amount of ozone is required, making the process expensive.

2.3.2. Acid hydrolysis

Concentrated acids such as H₂SO₄ and HCl have been used to treat lignocellulosic materials. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic,

corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible [33].

Dilute acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. The dilute sulfuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis [34]. At moderate temperature, direct saccharification suffered from low yields because of sugar decomposition. High temperature in dilute acid treatment is favorable for cellulose hydrolysis [9]. Recently developed dilute acid hydrolysis processes use less severe conditions and achieve high xylan to xylose conversion yields. Achieving high xylan to xylose conversion yields is necessary to achieve favorable overall process economics because xylan accounts for up to a third of the total carbohydrate in many lignocellulosic materials [35]. There are primarily two types of dilute acid pretreatment processes: high temperature ($> 160^{\circ}\text{C}$), [36][37] and low temperature ($< 160^{\circ}\text{C}$), [34][38]. Although dilute acid pretreatment can significantly improve the cellulose hydrolysis, its cost is

usually higher than some physico-chemical pretreatment processes such as steam explosion or AFEX. A neutralization of pH is necessary for the downstream enzymatic hydrolysis or fermentation processes.

2.3.3. Alkaline hydrolysis

Some bases can also be used for pretreatment of lignocellulosic materials and the effect of alkaline pretreatment depends on the lignin content of the materials[9][39]. Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure [9] [40]. Ammonia was also used for the pretreatment to remove lignin [41].

2.3.4. Oxidative delignification

Lignin biodegradation could be catalyzed by the peroxidase enzyme with the presence of H_2O_2 . The pretreatment of cane bagasse with hydrogen peroxide greatly enhanced its susceptibility to enzymatic hydrolysis. [42]

2.3.5. Organosolv process

In the organosolv process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4) is used to break the internal lignin and hemicellulose bonds. The

organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol [43][44]. Organic acids such as oxalic, acetylsalicylic and salicylic acid can also be used as catalysts in the organosolv process [45]. Usually, a high yield of xylose can be obtained with the addition of acid. Solvents used in the process need to be drained from the reactor, evaporated, condensed and recycled to reduce the cost. Removal of solvents from the system is necessary because the solvents may be inhibitory to the growth of organisms, enzymatic hydrolysis, and fermentation.

2.4. Biological pretreatment:

In biological pretreatment processes, microorganisms such as brown-, white- and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials [46]. Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials [39]. The advantages of biological pretreatment include low energy requirement and mild environmental conditions. However, the rate of hydrolysis in most biological pretreatment processes is very low.[47]

Enzymatic hydrolysis of cellulose is carried out by cellulase enzymes which are highly specific [48]. The products of the hydrolysis are usually reducing sugars including glucose. Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45– 50 °C) and does not have a corrosion problem [49]. Both bacteria and fungi can produce cellulases for the hydrolysis of lignocellulosic materials.

Concluding remarks:

Several pretreatment methods have been presented for lignocelluloses in order to improve ethanol or biogas production. All these methods should make the lignocelluloses available to the enzymatic attack, where crystallinity of cellulose, its accessible surface area and protection by lignin and hemicellulose are the main factors in order to obtain an efficient hydrolysis. In addition, the efficient utilization of the hemicelluloses is an opportunity to reduce the cost of ethanol production. Diverse advantages have been reported for most of the pretreatment methods, which make them interesting for industrial applications. Some of these methods are highly expensive while some other methods such as biological pretreatment are extremely slow. Furthermore, some technological factors such as energy balance, solvent recycling and corrosion, as well as environmental factors such as wastewater treatment, should be carefully considered for the selected method.

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