

Fermentation of Crop Residues and Fruit Wastes for Production of Ethanol and Its Pretreatment Methods: A Review

Alemayehu Gashaw¹ & Tewodros Getachew¹

Department of Chemistry, Faculty of Natural and Computational Sciences, BuleHora University, BuleHora, Ethiopia

Corresponding Author's Email: alexgashaw@gmail.com

Abstract:

Ethanol is a renewable resource of energy and is potentially cleaner alternative to fossil fuels. The search for alternative and renewable source of fuels especially for transportation has taking a centre stage across the world due to its ecofriendliness (minimize emission of greenhouse gases) and environmental sustainability. Cellulosic plant materials like fruit wastes and crop residues are mainly composed of cellulose, hemicellulose and lignin, the cheapest source of fermentable sugars. They are attractive feed stocks for bioethanol production due to their cost effectiveness, renewability and abundance. The pretreatment methods can significantly enhance hydrolysis of biomass and thus enhance ethanol production. The present review focuses on various pretreatment methods based on composition of lignocellulosic biomass and production of ethanol by fermentation.

Key words:

Ethanol; Fruit waste; Lignocellulosic materials; Pretreatment

Introduction

Global depletion of fossil fuels, rising fuel prices, environmental concerns, and pressures for oil independence are creating a

strong market for biofuels. In addition, demand of the energy increases with the increase of the world population and urbanization and thus, development of bioenergy as alternative energy might help to reduce these problems (Hossain *et al.*, 2011). The first large scale use of ethanol as a fuel happened during the early 1900s when petroleum supplies in Europe were short. Though ethanol is conventionally produced from petroleum by-products, bio-ethanol can alternatively be produced by fermentation technology using renewable raw material (Shilpa *et al.*, 2013).

Increase on world's energy demand and the progressive depletion of oil reserves motivate the search for alternative energy resources, especially for those derived from renewable materials such as biomass. Global concern about climate change and the consequent need to diminish greenhouse gases emissions have encouraged the use of bioethanol as a gasoline replacement or additive (Dias *et al.*, 2009).

Liquid bio-fuels are receiving increasing attention worldwide as a result of the growing concerns about oil security of supply and global climate change. In most developing countries, the emerging bio-fuels

industry is perceived as an opportunity to enhance economic growth and create or maintain jobs, particularly in rural areas. The liquid bio-fuels market is shared mainly between bio ethanol and biodiesel, with more than 85% market share for the former in 2005. The main advantage of bio ethanol is the possibility to blend it in low proportions with gasoline (5 to 25% bio ethanol by volume) for use, without any significant change, in internal combustion engines (Osunkoya and Okwudinka, 2011).

Ethanol is mostly used as fuel and has become an alternative of renewable energy source now-a-days. Ethanol is an eco-friendly alternative to petroleum-based fuel as it has fewer greenhouse gas emissions. The production of ethanol is growing day by day at a great extent for its versatile application and demand. As the supply and price of oil and gas worldwide has become a major problem, ethanol is taking place as an alternative. Worldwide ethanol production as fuel reached 32.35 billion gallons in 2012. Ethanol is obtained by the fermentation process where the sugar content in juices and honey of crops are transformed into alcohol using yeast. (Ruhul Amin *et al.*, 2013). As energy demand increases the global supply of fossil fuels, it causes harm to human health and contributes to the greenhouse gas (GHG) emission. Hahn-Hagerdal (13) alarmed to the society by seeing the security of oil supply and the negative impact of the fossil fuel on the environment, particularly on GHG emissions. The reduction of GHG pollution is the main advantage of utilizing biomass conversion into ethanol (14). Ethanol contains 35% oxygen that helps complete combustion of fuel and thus reduces particulate emission that pose health hazard to living beings (Naiman *et al.*, 2011).

Significant quantities of decaying fruits are discarded as wastes which cause real

environmental problems, these can be used as potential feedstock for bioethanol production and this could also be an attractive alternate for disposal of the polluting residues (Girishet *al.*, 2014). Citrus fruits are one of the major fruit crop cultivated worldwide. In the citrus processing industry citrus peel is the major solid by-product and comprises around 50% of the fresh fruit weight and can be utilized as substrate for bioethanol production. There are major limitations to efficient ethanol production from agricultural residues. These limitations include the close physical and chemical association between lignin and plant cell wall polysaccharides together with cellulose crystallinity. Lignin forms a protective shield around ethanol; the cellulose must be readily available for cellulose enzyme. Thus by removing the lignin, the cellulose becomes vulnerable to enzymes and allows the yeast to convert the glucose into ethanol during fermentation. Therefore, the pretreatment method can be applied to degrade the lignin in the peel residue, decrease cellulose crystallinity and increase the surface area for enzymatic activity. (Prasad Singh, 2014).

Production of ethanol from sugary materials is easier than lignocellulosic materials, since it requires additional technical challenges such as pretreatment (Girishet *al.*, 2014). Lignocellulosic biomass, the most abundant, high polysaccharides (cellulose and hemicellulose) content and low-cost biomass worldwide are the raw materials for production of fuel ethanol (Xuan, 2010). For transformation of lignocellulosics into bioethanol, the steps involved are procurement of lignocellulosics, pretreatment, hydrolysis to derive sugars their fermentation bioethanol and its dehydration. In general, ethanol production from lignocellulosic feedstocks grouped as:

- ✓ Crop Residues (cane bagasse, corn stover, wheat straw, rice straw, rice hulls, barley straw, sweet sorghum bagasse, olive stones and pulp); and
- ✓ Municipal Solid Wastes (MSW) such as fruit wastes.(Verma *et al.*, 2011)

This review paper clearly describes the ethanol production process and the pretreatment methods used for ethanol production.

Properties of Ethanol

Ethanol or ethyl alcohol, $\text{CH}_3\text{CH}_2\text{OH}$, has been described as one of the most exotic synthetic oxygen-containing organic chemicals because of its unique combination of properties as a solvent, a germicide, a beverage, antifreeze, a fuel, a depressant, and especially because of its versatility as a chemical intermediate for other organic chemicals. Ethanol, also known as ethyl alcohol or grain alcohol, is a volatile, flammable, colorless chemical compound. It is a monohydric primary alcohol and it boils at 78.5°C . It is miscible (i.e., mixes without separation) with water in all proportions and is separated from water only with difficulty; ethanol that is completely free of water is called absolute ethanol. Ethanol forms a constant-boiling mixture, or azeotrope, with water that contains 95% ethanol and 5% water and that boils at 78.15°C . Ethanol is a psychoactive agent and it produces a variety of physiological and behavioral effects (Gaur, 2006).

Ethanol production process

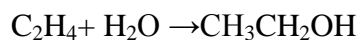
Ethanol was first prepared synthetically in 1826, through the independent effort of Henry Hennel in Britain and S.G in France. Michael Faraday prepared ethanol by the acid-catalysed hydration of

ethylene in 1828, in a process similar to that used for industrial synthesis of ethanol today (Shilpa *et al.*, 2013).

Ethanol is primarily produced from two major categories, catalytic hydration of ethylene (Synthetic ethanol) and bio-fermentation of agricultural feed stocks such as fruits, vegetables, and cereals (Balasubramanian K. *et al.*, 2011).

Ethylene hydration

Ethanol for use as industrial feedstock is most often made from petrochemical feedstocks, typically by the acid-catalyzed hydration of ethylene, represented by the chemical equation



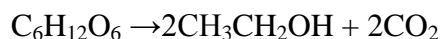
The catalyst is most commonly phosphoric acid, adsorbed onto a porous support such as diatomaceous earth or charcoal; this catalyst was first used for large scale ethanol production by the Shell Oil Company in 1947 (Gaur, 2006).

Fermentation

Fermentation is a bio-chemical process wherein commercial products are obtained through the activity of micro-organism. The chemical changes are brought about in an organic substrate through the action of enzymes by micro-organism. It is directly caused by the life processes of minute organisms. Micro-organisms, which include; bacteria, yeast and moulds, feeds on the organic materials, if they are supplied with the necessary energy, along with other needed nutrients, this process is of utmost interest to the manufacturer. These micro vegetative organisms will not only grow and multiply, but will change the substrate into other chemical substances (Misau *et al.*, 2012).

Bioethanol is produced from the fermentation of sugars obtained from biomass. Bioethanol feedstock can contain either sucrose (e.g. sugarcane, sugar beet) or starch (e.g. corn, wheat) or be a lignocellulosic material (e.g. sugarcane bagasse, wood and straw). Sugarcane is so far the most efficient raw material for bioethanol production: the consumption of fossil energy during sugarcane processing is much smaller than that of corn. Furthermore, optimization of bioethanol production process from sugarcane is still possible, and significant reduction of energy consumption can be achieved (Diasaet *al.*, 2009).

Ethanol for use in alcoholic beverages, and the vast majority of ethanol for use as fuel, is produced by fermentation: when certain species of yeast (most importantly, *Saccharomyces cerevisiae*) metabolize sugar in the absence of oxygen, they produce ethanol and carbon dioxide. The overall chemical reaction conducted by the yeast may be represented by the chemical equation



The process of culturing yeast under conditions to produce alcohol is referred to as brewing. Brewing can only produce relatively dilute concentrations of ethanol in water; concentrated ethanol solutions are toxic to yeast. The most ethanol tolerant strains of yeast can survive in up to 25% ethanol (by volume). In order to produce ethanol from starchy materials such as cereal grains, the starch must first be broken down into sugars (Gaur, 2006; Gupta and Demirbas, 2010).

Production of ethanol from lignocellulosic materials

Lignocellulose consists of three main components: cellulose, hemicellulose and

lignin, the first two being composed of chains of sugar molecules. These chains can be hydrolysed to produce monomeric sugars, some of which can be fermented using ordinary baker's yeast. To attain economic feasibility a high ethanol yield is necessary. However, producing monomer sugars from cellulose and hemicellulose at high yields is far more difficult than deriving sugars from sugar- or starch-containing crops, e.g. sugarcane or maize. Therefore, although the cost of lignocellulosic biomass is far lower than that of sugar and starch crops, the cost of obtaining sugars from such materials for fermentation into bioethanol has historically been far too high to attract industrial interest. For this reason, it is crucial to solve the problems involved in the conversion of lignocellulose to sugar and further to ethanol. However, the heterogeneity in feedstock and the influence of different process conditions on microorganisms and enzymes makes the biomass-to-ethanol process complex (Galbe and Zacchi, 2002; Ali, 2011).

Ethanol can be produced from lignocellulosic materials in various ways. The main features of the different ethanol processes are outlined in Figure 1. All processes comprise the same main components: hydrolysis of the hemicellulose and the cellulose to monomer sugars, fermentation and product recovery and concentration by distillation. The main difference between the process alternatives is the hydrolysis steps, which can be performed by dilute acid, concentrated acid or enzymatically. Some of the process steps are more or less the same, independent of the hydrolysis method used. For example, enzyme production will be omitted in an acid-hydrolysis process; likewise, the recovery of acid is not necessary in an enzyme-hydrolysis process.

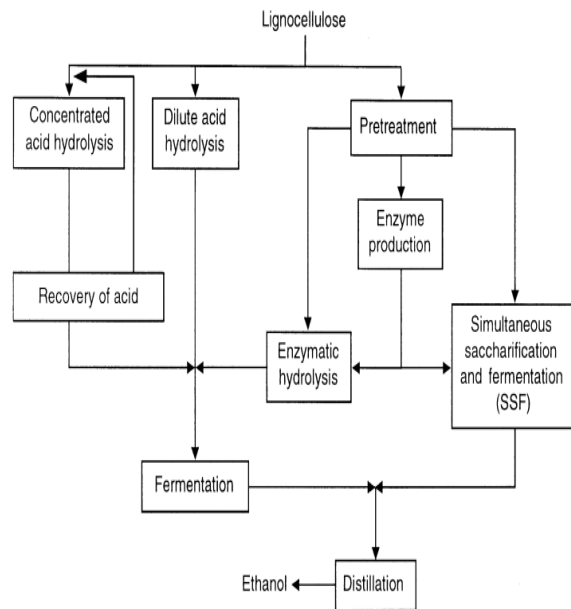


Figure 1: Production of ethanol from lignocellulosic materials (Galbe and Zacchi, 2002).

Raw materials for bioethanol production

Bioethanol is produced from the fermentation of sugars obtained from biomass. Bioethanol feedstock can contain either sucrose (e.g. sugarcane, sugar beet) or starch (e.g. corn, wheat) or be a lignocellulosic material (e.g. sugarcane bagasse, wood and straw) (Diasa, et al., 2009). Many different feedstock sources can be used for ethanol production is shown in Figure 4. Two examples of feedstock for ethanol production are sugar beets and sugar cane which contain high percentages of sugar. Sugars can be easily fermented. Corn, wheat, barley, rye and other cereals are typical feedstock containing starch in their kernels. Starch can relatively easily be converted into sugar and then into ethanol. In the USA and Europe, ethanol is manufactured mainly from maize and grain (Khan and Dwivedi, 2013).

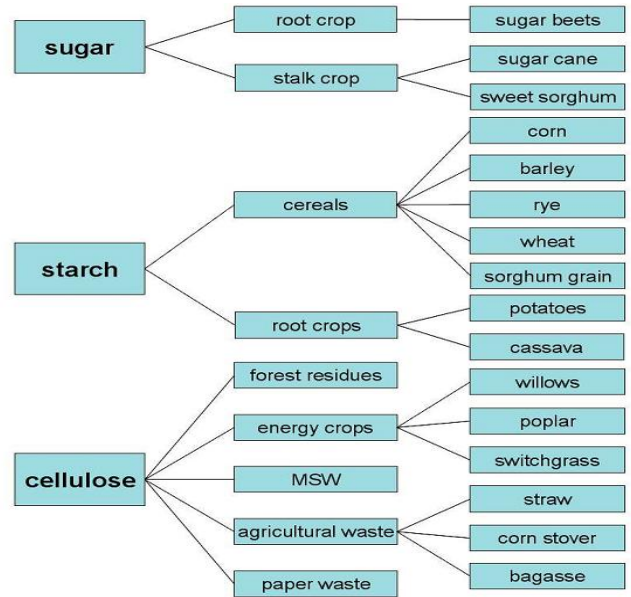


Figure 2: Types of feedstock for ethanol production with their examples (Khan and Dwivedi, 2013).

Pretreatment

Pretreatment of lignocellulosics aims to decrease crystallinity of cellulose, increase biomass surface area, remove hemicellulose, and break the lignin barrier (Verma et al., 2011). Pretreatment is required to alter the structures of cellulosic biomass to make more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars in a rapid way with the concomitant more yield and to cellulase producing microorganisms. There are several ways to increase the digestibility of cellulose before it is exposed to enzyme or microbial conversion. Pretreatment methods include mechanical, physical, chemical or biological pretreatment, as well as the combination of these methods (Patel et al., 2007; Verma et al., 2011).

Pretreatment uses various techniques, including ammonia fiber explosion, chemical treatment, biological treatment, and steam explosion, to alter the structure of cellulosic biomass to make cellulose more

accessible. Then, acids or enzymes can be used to break down the cellulose into its constituent sugars. Enzyme hydrolysis is widely used to break down cellulose into its constituent sugars. The goal of pretreatment in biomass-to-biofuels conversion is depicted in Figure 5.

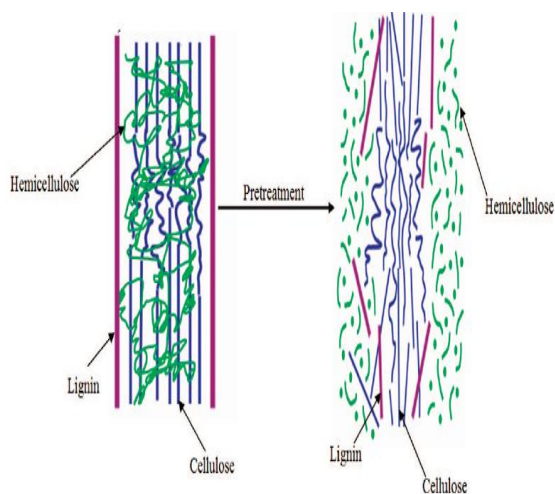


Figure 3. Schematic of the role of pretreatment in the conversion of biomass to fuel (Kumar *et al.*, 2009).

Physical pretreatment

Mechanical size reduction

Raw materials of bioethanol production can be technically reduced to smaller size by various processes comprised of fragmenting, pulverizing and granulating processes (Mukherjee *et al.*, 2014). This reduces cellulose crystallinity and improves the efficiency of downstream processing. Wet milling, dry milling, vibratory ballmilling and compression milling are usually done. The power input for mechanical comminution of agricultural materials depends on the initial and final particle sizes, moisture content and on the nature of waste (hardwood, softwood, fibrous, etc) being handled. Size reduction may provide better results but very fine particle size may impose negative

effects on the subsequent processing such as pretreatment and enzymatic hydrolysis. It may generate clumps during the subsequent steps involving liquid and may lead to channeling. Specific energy consumption also increases (Sarkar *et al.*, 2012). 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 (Sun and Cheng, 2002).

Pyrolysis/thermal cracking

Pyrolysis— Pyrolysis takes place at elevated temperatures. This progression mortifies cellulose briskly into H_2 , CO , and enduring char. The recuperated solution compiled of glucose obtained after the severance of char, can be ultimately fermented for manufacturing ethanol (Mukherjee *et al.*, 2014).

Physicochemical pretreatment

Steam explosion or autohydrolysis

Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials. 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 (corresponding pressure 0.69–4.83 MPa) 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. When steam is allowed to expand within the lignocellulosic matrix it separates the individual fibers. Steam-explosion pretreatment is economically

attractive at high recovery of xylose (45-65%) (Sarkaret *al.*, 2012).

The factors that affect steam explosion pretreatment are steam temperature, residence time in the reactor, particle size, moisture content and the catalyst concentration. Recent studies indicate that lower temperature and longer residence time are more favorable (Vermaet *al.* 2011).

The advantages of steam explosion pretreatment include the low energy requirement compared to mechanical comminution and no recycling or environmental costs. The conventional mechanical methods require 70% more energy than steam explosion to achieve the same size reduction. 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 (Sun and Cheng, 2002).

Ammonia Fiber Explosion (AFEX)

Ammonia fiber explosion is another type of physico-chemical pretreatment in which lignocellulosic materials are exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is swiftly reduced. The concept of AFEX is similar to steam explosion (Sun and Cheng, 2002). The use of ammonia as a swelling agent for water ingress and disruption therefore does not cause depolymerisation of the cellulose/hemicellulose fraction, reducing the efficiency of conversion in the subsequent enzymatic hydrolysis. Increased efficiency is seen in low lignin content materials, therefore AFEX is advantageous for herbaceous and agricultural residues, with little produced in terms of inhibitory elements. AFEX also has

the benefit of reduced downstream processing prior to fermentation. The drawback of the process is the loss of efficiency in higher lignin content materials such as softwood chips and paper residues, reducing flexibility in the bio refinery feedstocks. In addition, AFEX has difficulty in solubilising smaller fractions such as hemicellulose (Hayes, 2009).

The AFEX process requires efficient ammonia recovery to be economical due to the high cost of ammonia. A possible approach is to recover the ammonia after the pretreatment by evaporation. The major parameters influencing the AFEX process are ammonia loading, temperature, high pressure, moisture content of biomass, and residence time (Sarkaret *al.*, 2012).

Carbon Dioxide Explosion

Supercritical carbon dioxide has been considered as an extraction solvent for non-extractive purposes, due to several advantages such as availability at relatively low cost, non-toxicity, nonflammability, easy recovery after extraction, and environmental acceptability (Taherzadeh and Karimi, 2008).

In addition to steam and ammonia explosion pretreatment, CO₂ explosion is also used for pretreatment of lignocellulosic materials. However, CO₂ explosion is more cost effective than ammonia explosion and does not cause the formation of inhibitors as in steam explosion. Conversion yields are higher compared to the steam explosion method (Sarkaret *al.*, 2012).

In attempts to develop improved lignocellulose pretreatment techniques, the idea of using supercritical CO₂ explosion, which would have a lower temperature than steam explosion was developed. It was hypothesized that, because CO₂ forms

carbonic acid when dissolved in water, the acid increases the hydrolysis rate. Carbon dioxide molecules are comparable in size to water and ammonia and should be able to penetrate small pores accessible to water and ammonia molecules. Carbon dioxide was suggested to be helpful in hydrolyzing hemicellulose as well as cellulose. Moreover, the low temperature prevents any appreciable decomposition of monosaccharides by the acid. Upon an explosive release of the carbon dioxide pressure, the disruption of the cellulosic structure increases the accessible surface area of the substrate to hydrolysis (Kumar *et al.*, 2009; Taherzadeh and Karimi, 2008).

Chemical pretreatment

Chemical pretreatment method involves acid and alkali pre-treatments, which are dominant methods for treating cellulosic biomass. Biochemically the difference between the two methods is the solubilising of the enzyme inhibitor. In acid pretreatment, solubilisation of the hemicellulose fraction is observed whereas in alkali pretreatment, the target is the lignin fraction as illustrated in Figure 6. Chemical pretreatment method also involves ammonia, organic solvent, SO₂, CO₂ or other chemicals. These methods are easy in operation and have good conversion yields in short span of time.

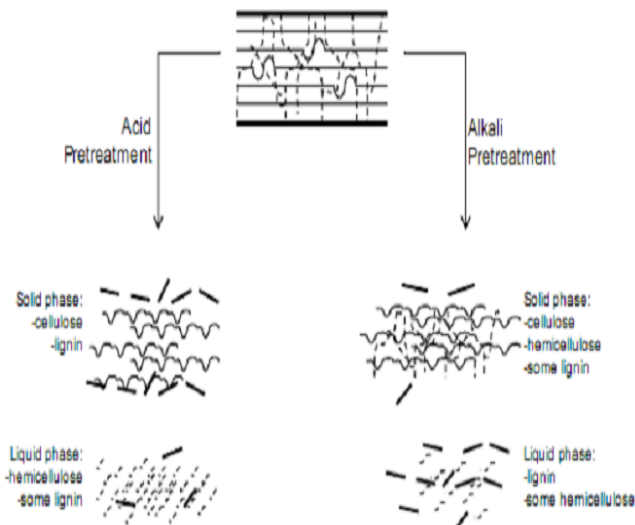


Figure 4: Comparison of alkali and acid pretreatment methods and fractionation of ligno-cellulosic feedstocks (Keshwani, (2009).

Acid pretreatment

Acid pretreatment is considered as one of the most important techniques and aims for high yields of sugars from lignocellulosics. There are two types of acid hydrolysis process commonly used- dilute and concentrated acid hydrolysis. The dilute acid process is conducted under high temperature and pressure and has reaction time in the range of seconds or minutes. The concentrated acid process uses relatively mild temperatures, but at high concentration of sulfuric acid and a minimum pressure involved (Chandele *et al.*, 2007). Unlike alkali pretreatment, acid pretreatment does not disrupt lignin but is thought to work by breaking down hemicellulose and disrupting ether bonds between lignin and hemicellulose. Acid pretreatment is typically used in combination with heat (Montgomery and Bochmann, 2014).

Sulfuric acid is widely used for acid pretreatment among various types of acid such as hydrochloric acid, nitric acid and

phosphoric acid. Acid pretreatment can utilize either dilute or concentrated acids to improve cellulose hydrolysis. The acid medium attacks the polysaccharides, especially hemicelluloses which are easier to hydrolyze than cellulose. However, acid pretreatment results in the production of various inhibitors like acetic acid, furfural and 5 hydroxymethyl furfural. These products are growth inhibitors of microorganisms. Hydrolysates to be used for fermentation therefore need to be detoxified. Sarkaret *al* (2012) describes higher hydrolysis yield from lignocellulose which are pretreated with diluted H_2SO_4 compared to other acids. There are primarily two types of dilute acid pretreatment processes: high temperature (T greater than $160\text{ }^\circ\text{C}$), continuous-flow process for low solids loading (5–10% [weight of substrate/weight of reaction mixture]), and low temperature (less than $160\text{ }^\circ\text{C}$), batch process for high solids loading (10–40%). 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 (Sun and Cheng, 2002).

Alkaline pretreatment

Alkali pretreatment refers to the application of alkaline solutions such as NaOH, $Ca(OH)_2$ (lime) or ammonia to remove lignin and a part of the hemicellulose, and efficiently increase the accessibility of enzyme to the cellulose (Taherzadeh and Karimi, 2008). The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components, for example, lignin and other hemicellulose. The porosity of the lignocellulosic materials increases with the removal of the crosslinks.

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 (Sun and Cheng, 2002).

Pretreatment can be performed at low temperatures but with a relatively long time and high concentration of the base. For instance, when soybean straw was soaked in ammonia liquor (10%) for 24 h at room temperature, the hemicellulose and lignin decreased by 41.45% and 30.16% respectively. However, alkaline pretreatment was shown to be more effective on agricultural residues than on wood materials (Taherzadeh and Karimi, 2008).

Ozonolysis

For the humilation of the lignin and hemicellulose portions from lignocellulosic raw materials such as wheat straw, bagasse, green hay, peanut, pine, cotton straw, and poplar sawdust, ozone can be exploited (Mukherjee *et al.*, 2014). 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. 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. However, a large amount of ozone is required, making the process expensive (Sun and Cheng, 2002).

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. About 50% lignin and most hemicelluloses were solubilized by 2% H_2O_2 at 30 °C within 8 hr, and 95% efficiency of glucose production from cellulose was achieved in the subsequent saccharification by cellulase at 45 °C for 24 hr (Sun and Cheng, 2002).

Wet oxidation

In wet oxidation, the feedstock material is treated with water and either by air or oxygen at temperatures above 120 °C. The water is added to the biomass at a ratio of 1 L per 6 g of biomass. The transfer of hemicelluloses from solid phase to the liquid phase is promoted in this technique. It does not hydrolyze the liberated hemicellulose molecules. The products of hemicellulose hydrolysis during wet oxidation are sugar oligomers. There have been several studies on wet oxidation as a pretreatment strategy using different substrates (Sarkar et al., 2012).

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. It is an alternative method for the delignification of lignocellulosic materials. The organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol. Organic acids such as oxalic, acetylsalicylic and salicylic acid can also be used as catalysts in the organosolv process. At high temperatures (above 185 °C),

the addition of catalyst was unnecessary for satisfactory delignification. 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 (Mukherjee et al., 2014; Sarkar et al., 2012; Sun and Cheng, 2002).

Biological pretreatment

Degradation of the lignocellulosic complex to liberate cellulose can be brought about with the help of microorganisms like brown rot, white rot and soft rot fungi. Biological pretreatment renders the degradation of lignin and hemicellulose and white rot fungi seem to be the most effective microorganism. Brown rot attacks cellulose while white and soft rots attack both cellulose and lignin. Cellulase-less mutant was developed for the selective degradation of lignin and to prevent the loss of cellulose but in most cases of biological pretreatment the rate of hydrolysis is very low. This method is safe and energy saving due to less mechanical support. It needs no chemicals but low hydrolysis rates and low yields impede its implementation. Biological pretreatment of bamboo culms with white rot fungi has been performed at low temperature (25 °C) (Sankar et al., 2012).

Enzymatic hydrolysis

Hydrolysis of cellulosic materials can be catalyzed by a class of enzymes known as cellulases. These enzymes are mainly produced by fungi, bacteria, and protozoans that catalyze the cellulolysis or hydrolysis of cellulose. At least three major groups of enzymes including exo-glucanase, endo-glucanase and β -

glucosidase are involved in depolymerization of cellulose to glucose. β -glucosidase catalyzes cleavage of cellobiose, which plays a significant role in the hydrolysis process, since cellobiose is an end-product inhibitor of many cellulases including both exo- and endo-glucanases. β -glucosidase, in turn, is inhibited by glucose and, therefore, enzymatic hydrolysis is sensitive to the substrate concentration. In addition to substrate concentration, pretreatment of cellulosic materials and hydrolyzing conditions such as temperature and pH are among factors influencing the effectivity of enzymatic hydrolysis. Most cellulase enzymes show an optimum activity at temperatures and pH in the range of 45-55°C and 4-5, respectively (Talebnia, 2008).

Therefore, enzymatic hydrolysis is advantageous because of its low toxicity, low utility cost and low corrosion compared to acid or alkaline hydrolysis. Moreover, no inhibitory by-product is formed in enzymatic hydrolysis. However, enzymatic hydrolysis is carried out by cellulase enzymes that are highly substrate specific. Here cellulase and hemicellulase enzymes cleave the bonds of cellulose and hemicellulose respectively. Cellulose contains glucan and hemicellulose contains different sugar units such as mannan, xylan, galactan and arabinan (Sankaret *et al.*, 2012).

Uses of ethanol

a. As a solvent

Industrially, ethanol is mainly used as a carrier solvent in inks and coatings. Consumer applications include the use of ethanol in professional cosmetic formulations such as hair setting sprays and colorants, as well as in consumer cleaning and detergent preparations, for example spray cleaners used in kitchens and

bathrooms. Most perfumes consist of blends of predominantly natural essences in an ethanol base. The essences themselves are often extracted from flowers and barks using ethanol as the process solvent. Ethanol is also found in automotive deicing products. Ethanol is also used in pharmaceutical and personal care products. Preparations such as mouthwashes, and cough and cold medicines, are formulated with up to 30% ethanol. Ethanol is also used as an active biocidal product (Diasaet *et al.*, 2009).

b. As an intermediate for the production of synthetic chemicals

Bioethanol may also be used as raw material for the production of different chemicals, thus driving a full renewable chemical industry (Diasa *et al.*, 2009). As a reactive chemical, ethanol in common with all alcohols reacts with acids to produce esters. Examples include ethyl acrylate, which is used as a reactive diluent in specialised coatings, and ethyl acetate, which is a widely used solvent in paint and coating formulations. Ethanol is used in the production of ethylamines, which in turn are reactive industrial chemicals used in downstream speciality applications including agrochemicals and pharmaceuticals. It can also be used to make ethoxypropanol, an increasingly used glycol ether solvent in coating formulations (CEFIC, 2003).

Use of ethanol in fuel

The use of ethanol as a gasoline fuel additive as well as transportation fuel helps to alleviate global warming and environmental pollution (Patel *et al.*, 2007).

Ethyl alcohol as "the fuel of the future" was presented by Henry Ford for the first time. In 1925, he told the New York Times: "The fuel of the future is going to come from fruit

like that sumac out by the road, or from apples, weeds, sawdust – almost anything... There is fuel in every bit of vegetable matter that can be fermented. There's enough alcohol in one year's yield of an acre of potatoes to drive the machinery necessary to cultivate the fields for a hundred years." However, fossil fuels were predominantly used for automobile transportation throughout the last century, obviously due to their lower production cost. As an automotive fuel, hydrous ethanol can be used as a substitute for gasoline in dedicated engines. Anhydrous ethanol, on the other hand, is an effective octane booster when mixed in blends of 5 to 30% with no engine modification requirement (Licht, 2006).

GHG emission reduction potential

Researchers at the University of California at Berkeley estimated that on a life-cycle basis, cellulosic ethanol could lower GHG emissions by 90 percent relative to petroleum-based gasoline. Other analyses have shown that cellulosic ethanol produced using certain feedstocks could be carbon negative, which means that more carbon dioxide (CO₂) is removed from the atmosphere than is emitted into the atmosphere over the entire life-cycle of the product. However, these studies do not include estimates of emissions due to indirect land use change, which can affect GHG emission profiles significantly (Climate tech book, 2009).

Conclusion

Lignocellulosic biomass has been used as one of the main resources for economically attractive bioethanol production. Though theoretical ethanol yields from sugar and starch (g ethanol/g substrate) are higher than from lignocellulose, these conventional sources are insufficient for worldwide

bioethanol production. As regards conversion technology the hindrances are biomass processing, proper and cost effective pretreatment technology to liberate cellulose and hemicellulose from their complex with lignin. In respect of the hydrolysis process the challenge is to achieve an efficient process for depolymerization of cellulose and hemicellulose to produce fermentable monomers with high concentration. In this aspect enzymatic hydrolysis may be the most potent alternative process for saccharification of complex polymer. It is possible to manufacture ethanol (a relatively low-cost alternative fuel) from fruit wastes by fermentation and it is considered to be better for the environment than gasoline.

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