

Various Pretreatment Technologies for Bioethanol Production: A Review

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Abstract:-- Due to increase in population and industrialization, all over the globe the demand for fossil based fuel is constantly increasing. This increase in demand for non-renewable fossil based fuel is exhausting the reservoirs of oil as well as possesses severe threats to environment. Thus energy sector have shifted its focus from non-renewable fossil based fuel to renewable biofuels like biogas, bioethanol, biodiesel, etc., contributing towards sustainable development and overcome the threats to environment. Among these biofuels, bioethanol is substituting source of energy receiving special attention over the globe due to exhaustion of non-renewable fossil fuels and has some significant advantages such as its capability to replace gasoline, to achieve the carbon emission goals under Kyoto Protocol and can be used as transportation fuel with minimal or no modifications in the existing vehicular engines. This bioethanol is produced using lignocellulosic materials and biomass like municipal solid waste, crops, etc. But crops cannot meet the global requirements of bioethanol production considering food v/s fuel debate. So, the lignocellulosic substances such as agricultural wastes, kitchen waste, municipal solid waste etc., are captivating raw material for bioethanol production. Agricultural wastes and kitchen wastes are not only cost effective but are also renewable and ample in quantity. Bioethanol from lignocellulosic material could be a promising technology though the process has several provocations and limitations such as handling and transport of the biomass and coherent pre-treatment methods for total delignification of lignocellulosic materials. Concentrations of fermentable sugars after enzymatic saccharification or enzymatic hydrolysis can be increased by considering appropriate pre-treatment methods, also improving the efficiency of the process. Converting glucose as well as xylose to bioethanol needs some new technologies, to make the whole process cost effective. This paper reviews various pre-treatment methods available for lignocellulosic materials to be further hydrolyzed and fermented to produce bioethanol.

Index Terms—Bioethanol, Pre-treatment, Lignocellulosic, Fermentation..

I. INTRODUCTION

Globalization, industrial revolution and increasing population have raised the demands for fossil fuel. In present scenario, our economy highly depends on energy sources which are generally derived from fossils like coal, natural gas, oil, etc [1]. It helps to satisfy our need by generation of electricity, fuel production, and producing other goods. Our dependency on such non-renewable sources not only leads reserves of oil to an exhaustion stage but also possess threat to our environment, causing high pollution potential, increasing levels of green house gases, increasing global warming and drastic change in climatic conditions.

To resolve these problems and contributing towards sustainable development, highlights the use of renewable resources like wind, water, biomass, geothermal heat and sun for energy focused industries while biomass as an alternative for near future for fuel and chemical industries. Generating and utilizing biofuels like biogas, biodiesel, bioethanol, etc., can be the key solution to these non-renewable fossils. A list of environmental and economical advantages is declared for bio-fuels.

Among these biofuels, production of bioethanol is a probable source for low cost biofuel generation and potential to blend with diesel, gasoline, petrol with or without modification in vehicular engines [7].

Under Ethanol Blended Petrol Program launched by Government of India in January 2003, it is made compulsory to blend 5 % ethanol (E5) to drive automobiles. By 2017 in India, it has been forecasted to utilize petrol with up to 20% blend of bioethanol and biodiesel. In the world, India with China and Thailand generates and grants 5% of production of bioethanol [3]. Across the globe, most familiar blend is 90% of petrol with 10% ethanol recognized as E10 but the vehicles having adaptable and modified engines can run on the blends upto 15% petrol and 85% of ethanol recognized as E85 [4]. When bioethanol is blended with petrol in lower amount, it results in increased octane number of the fuel and oxygen content of the fuel initiating to clean burning of the fuel with low emissions [5].

In India, for the production of ethanol molasses of sugar cane is the major raw material. But the increased cost and shortage of supply is the restraint for its use [8]. Whereas the lignocellulosic material used to produce

bioethanol are sufficiently available and provide the key solution to food v/s fuel debate. But these lignocellulosic materials require pre-treatment before undergoing hydrolysis and fermentation process and is the most expensive processing step [15]. Lignocellulosic materials are paper, wood chips, cotton, saw dust, municipal solid waste, crop residues like corn straw, etc [2, 3, and 6]. The lignocellulosic complex comprises of hemicellulose chains (15–35%) bounded in a matrix of cellulose (30–50%) and lignin (10–30%) [17]. the pre-treatment, breaks this matrix for reducing the degree of Crystallinity of the cellulose and increasing the fraction of amorphous cellulose that is suitable form for enzymatic attack. Also, delignification and hydrolyzation of hemicellulose should take place. The yield of cellulose hydrolysis after the pre-treatment of lignocellulosic material increases upto 90% of theoretical yield [16]. Thus, the objective of the pre-treatment, removal of lignin and hemicellulose and reducing the crystalline cellulose, and increases the porosity of the material. It also improves sugar formation further during enzymatic, and for subsequent process of hydrolysis and fermentation avoids the formation of inhibitors.

The financial resources for producing bioethanol depends on market for it's by products which is sent to distillers' dried grain which is served as animal food [2]. Thus, to make bioethanol viable and realistic, research in the area of pre-treatment of lignocellulosic material and enzymatic hydrolysis is carried on.

II. OBJECTIVES OF PRE-TREATMENT

For production of bioethanol being the first step, pretreatment plays important and crucial role in making biomass ready for conversion to C6 (cellulose) and C5 (hemicellulose) by enzymatic conversion or to oligomeric and monomeric sugars in few processes by direct hydrolysis of a structural carbohydrate [21]. For cellulose conversion process, pre-treatment is a crucial step for alteration of cellulose biomass structure making cellulose more approachable to enzymes which converts carbohydrate polymer to fermentable sugars. The objective is to break the seal of lignin and interrupt the crystalline cellulose structure. For conversion of cellulosic biomass to fermentable sugars the most expensive processing step is pre-treatment with cost as high as 3.72 Rupees per gallon of ethanol [15].

An effective pre-treatment is distinguished by various criteria. It circumvents the need for size reduction of biomass, prevention of fractions of hemicellulose, restricts the formation of degradation products which inhibits the fermentative micro-organisms growth and lowers the cost as well as minimizes the energy demand. Results of pre-treatment should be balanced against their influences on trade off between biomass cost, capital cost and operating cost and on the cost of downstream processing steps.

Advanced pre-treatment technologies should be developed that adjusts to characteristics of different biomass, controls the mechanism and minimizes the cost. The ultimate objective of pre-treatment is effective and systematic fractionation of lignocellulosic material to various streams containing value added products in concentration which makes recovery, utilization and purification economically feasible. The selection, optimization, design and process control technologies of pre-treatment matching appropriate method, its process configuration and biomass are enabled by predictive pre-treatment models.

III. PRE-TREATMENT

Pretreatment plays a crucial role in increasing the bioethanol yield by converting macroscopic structure, microscopic structure as well as the chemical composition of the feedstock. It is the fundamental step that is required when dealing with lignocellulosic feedstocks. An effective and efficient pretreatment technique is cost determining step in production of bioethanol as it can increase or decrease the capital and operating cost. It is the most challenging step as this technique solubilizes or separates cellulose, hemicellulose and lignin content of biomass. The recalcitrant components in the biomass is made accessible to the enzymes for saccharification step by solubilizing them using pretreatment method [1]. The lignin and cellulose content is covered by the chains of hemicellulose and this matrix is broken down to expose cellulose to cellulase enzyme for enzymatic hydrolysis using various pretreatment methods. The pretreatment method also helps in reducing degree of crystallinity of cellulose and converts it to more fraction of amorphous cellulose which is easily degraded by cellulase. Therefore, the pretreatment method reduces the degree of crystallinity of cellulose, breaks down lignin content to expose cellulose matrix to enzymes, increases amount of accessible sugar, reduces refractory contents, limits the

generation of toxic inhibitors, minimizes energy requirement, and decreases the overall cost [1, 3] The following section discusses the major types of pretreatment methods incorporated in the production of bioethanol.

II. PHYSICAL PRETREATMENT

The physical pretreatment is the method to reduce the size of the feedstock and rupture the lignin content using mechanical forces and thermal effects to make the cellulosic content and soluble sugars accessible for enzymatic hydrolysis. This pretreatment comprises of comminution, microwave oven irradiation pyrolysis [1] and electron beam irradiation [15].

(a) Comminution:

The basic pretreatment method is comminution which processes the lignocellulosic feedstock through methods like grinding, cutting, crushing and chipping. The method utilizes mechanical size reduction equipments like ball mill, hammer mill, vibratory ball mill, dry mill, wet mill, cutter mill, wet disk mill, etc., to obtain reduced size of the raw material. This method is used to decrease the concentration of crystalline cellulose and simultaneously increase the portion of amorphous cellulosic content. This method disintegrates the complex lignin and hemicellulosic matrix of the biomass to make it compatible for downstream operations. The result in more amounts of fine particles can reduce the efficiency by producing negative impacts like channeling and forming clumps in the process. The critical parameter in incorporating this pretreatment is the energy consumption by the above mentioned size reduction equipments [1]. For instance, if agricultural feedstock is utilized, then the energy consumption for mechanical reduction will be less as compared to forestry or woody biomass [3]. The energy consumed for reducing the wheat stover to 0.8 and 3.2 mm sizes using hammer mill were 51.6 and 11.4 kWh t⁻¹ respectively [1]. The chopping of banana peels and disinfecting using ethanol followed by fermentation using *Aspergillus Niger* yielded 6.540% bioethanol [9].

(b) Microwave oven irradiation:

This method uses the heating efficiency of microwaves produced in a microwave oven to produce thermal and non-thermal effects resulting in dissociation of chemical bonds of refractory materials in lignocellulosic feedstocks. This method can be proved to be a feasible method and is easy to operate. The thermal

effects produced in feedstock is generated due to vibration in the polar bonds of aqueous medium and biomass resulting to internal heat in the biomass matrix using microwave radiation. This results in formation of hotspot in the biomass matrix which causes an explosion effect to rupture the structure of refractory lignocellulosic material. Thermal pretreatment has an added advantage of releasing acetic acid which initiates autohydrolysis [1] Microwave pretreatment applied to feedstocks like bagasse and rice straw has been reported to yield 43 – 55 % of available sugar. The conversion of banana peel to bioethanol upto 6.289% using microwave oven irradiation pretreatment using microwave of power 160W for 5 minutes [10].

(c) Pyrolysis:

Pyrolysis also termed as destructive distillation can be an endothermic process that treats feedstock at temperature greater than 300 °C and decomposes the structure of biomass to hydrogen gas, carbon monoxide gas and residual char. The water present in the char contains great concentration of carbon which is leached out. This leached water contains glucose that can support the microbial growth and enhance bioethanol production [1]. About 80 – 85 % conversion of cellulose to sugar using mild acid leaching [1].

(d) Electron beam irradiation:

Irradiation degrades fraction of cellulose to fragile fibril, oligosaccharides having low molecular weight and cellobiose, due to favorable dissociation of the glucosidal bonds of the molecular chains of cellulose in the presence of lignin by irradiation. This method is expensive, requiring high energy and has industrial application problems. Using electron beam irradiation with accelerated electrons by a linear electron accelerator as a physical pre-treatment of milled and dried rice straw had the capacity to generate electron beams. Increases in yield is observed from 22.6% to 52.1% after enzymatic hydrolysis of untreated rice straw to that of electron beam irradiated rice straw after 132 hours of hydrolysis [15]. Scanning Electron Microscope and X-ray diffraction analysis shows physical changes to treated rice straw after this pre-treatment [14]. Generation of inhibitory substance during alkali or acid pre-treatment can be minimized or avoided as these methods do not involve the use of extreme temperatures.

III. PHYSICOCHEMICAL PRETREATMENT:

The physicochemical pretreatment combines physical and chemical treatments to reduce the recalcitrant material in biomass to reducing sugars. This pretreatment comprises of methods like steam explosion, ammonia fiber explosion, carbon dioxide explosion, etc.

(a) Steam explosion:

In the steam explosion method, steam is employed to rupture the structure of complex lignocellulosic substrate and make it accessible for attack by enzyme to carry on enzymatic hydrolysis. This method uses the energy of steam and is a feasible method for pretreatment containing high amount of refractory material as use of any type of catalyst is avoided. In the steam explosion method, the feedstock is exposed to high pressure steam generally at temperature between the range of 160 and 290 °C and pressure in the range 20-50 bar for few minutes. Then the reaction is stopped by reducing the pressure to atmospheric pressure. This sudden release of pressure results in expansion of steam in the inhomogeneous material of feedstock and disintegrates the material to fibers. These fibers are compatible to attack by cellulase enzyme. This pretreatment produces xylitol, levulinic acid and other alcohols. This pretreatment is economically feasible as it produces xylose upto 45-60% [1].

(b) Ammonia fiber explosion:

Ammonia fiber explosion (AFEX) utilizes both ammonia and steam to breakdown refractory material to individual fibers and is referred as an alkaline thermal pretreatment. The feedstock is exposed to high temperature and pressure for a few minutes and is followed by sudden release of pressure which causes the ammonia and steam to produce explosion effect and form individual fibers which are readily attacked by the enzymes. This pretreatment does not result in formation for inhibitory material in operation but is inefficient when employed for feedstocks having higher content of lignin. It also results in recovery of very small fraction of hemicellulose. But AFEX produces polymers that are susceptible to attack by enzymes for hydrolysis and does not directly reduce to sugar. The use of ammonia increases the cost of this method which can be reduced by recovering the ammonia by evaporation. The method reduces the residence from 5-10 minutes to 30 minutes. Cellulose and hemicellulose can be converted till 90% at optimum conditions [1]. Dilute Acid, sulphur dioxide, and Lime methods rendered higher 72 h digestibility than the

AFEX or soaking in aqueous ammonia for a fixed enzyme loading [23].

(c) Carbon dioxide explosion:

In the method of carbon dioxide explosion CO₂, high temperature, high pressure and sudden release of pressure results in disintegration of lignocellulosic feedstocks to fibers. It operates similarly as steam and ammonia fiber explosion method. But carbon dioxide explosion has an added advantage that it does not form toxic inhibitors that are formed in steam explosion. And it is more economically attractive and effective than AFEX as carbon dioxide is not too costly [1].

(d) Sulphur dioxide

Sulphite piping is well known technology using sulphur dioxide along with steam explosion. When combined with enzymatic hydrolysis it attains high yields of glucose and xylose. For a high enzymatic glucose yield, high temperature and less time is favorable where as low temperature and higher time is favorable for high enzymatic xylose yield. Using this pre-treatment, majority of hemicellulose carbohydrates is converted to soluble sugar by hydrolysis similar to dilute acid pre-treatment. Glucose is formed from small amount of cellulose and hemicellulose containing glucan but glucan remains in insoluble form further requiring enzymatic hydrolysis. Lignocellulosic material is first impregnated with sulphur dioxide and then high pressure steam heats upto targeted temperature in the pre-treatment reactor. Generally horizontal screw-feed reactor is used providing residence time of 10 minutes and temperature of 180°C [21]. 74.5% and 78.4% yield of glucose and xylose are obtained in this method [21].

(e) Liquid hot water treatment

Liquid hot water treatment method is a hydrothermal pretreatment technique that hydrolyzes hemicellulosic content in the biomass. In this process, at controlled pH the hot liquid water is compressed and is utilized to release high percentage of hemicellulose sugars [21]. The process occurs at 170 - 230°C temperature and under a pressure above 5 MPa for a period of 20 minutes [1]. But this releases refractory compounds which inhibit the microbial activity and hinder ethanol production. Still the treatment method is economically attractive as it recovers xylose by 88-98% [1]. Moreover, the method does not require use of chemicals which makes it environmentally reliable system [1]. Low reactor cost is

the advantage of this pre-treatment, but that is often equalized by lower monomeric xylose yield [21].

(f) Soaking in aqueous ammonia (SAA) pretreatment

Aqueous ammonia in a simple reactor configuration is used in this pre-treatment. A significant degree of delignification is attained by switch grass. In the aqueous solution 70% of the lignin is soluble; the remaining carbohydrate biomass which is insoluble in this aqueous solution is processed in subsequent enzymatic hydrolysis [21]. Soaking in aqueous ammonia is modified Ammonia recycle percolation method, which has a reaction temperature ranging between 150 to 180°C having 10% to 15% concentration of aqueous ammonia [21]. At high temperature, the biomass swells in aqueous ammonia and depolymerizes lignin, breaking the lignin-hemicellulose bonds without degradation of carbohydrates, enhancing the cellulose digestibility in enzymatic hydrolysis which is the subsequent downstream step. Whereas, soaking in aqueous ammonia process requires lower temperatures than the ammonia recycle process, typically ranging from 60–180°C [21]. In this method, for a time period (ranging from hours to days) the biomass is soaked in aqueous ammonia solution, and then treated at a high temperature and pressure for shorter residence time. It retains the hemicellulose in the biomass, eliminating separate processes of hemicellulose and cellulose sugars.

IV. CHEMICAL PRETREATMENT:

The chemical pretreatment techniques uses different chemicals like dilute acids, alkalis, organic solvents, ozone, SO₂, etc., to convert the lignocellulosic material to reducing sugars. For effective removal of lignin alkaline pretreatment, ozonolysis, peroxide and wet oxidation pretreatments are more efficient whereas solubilization of hemicellulose can be done effectively using dilute acid pretreatment [13]. However lignocellulosic structural components may have different effect of pretreatment based on the type of chemical used. This method provides high conversion in less residence time.

(a) Acid Pretreatment:

Acid pretreatment uses diluted or concentrated acids at temperature range 130 - 210°C to yield reducing sugars from lignocellulosic feedstocks to improve hydrolysis. Various types of acids are used for acid pretreatment namely sulphuric acid, nitric acid,

hydrochloric acid and phosphoric acid. These acids readily attack the hemicellulosic content which is easy to hydrolyze than the cellulosic portion. The acid pretreatment produces microbial growth inhibiting materials like furfural, acetic acid, etc., which need to be detoxified to reduce impact on downstream operations. 74% saccharification yield is obtained when wheat straw was subjected to acid pretreatment using 0.75% v/v of Sulphuric acid at 121 °C for 1 hour [1].

(b) Alkaline pretreatment:

In this method of pretreatment alkalis like potassium hydroxide, calcium hydroxide, sodium hydroxide, etc., are used to rupture the cell wall and temperature generally ranges between 120°C to 180°C [19]. Also constant supply of 100 psi pure oxygen is maintained [22]. These alkalis dissolve hemicellulose, silica and lignin, by breaking down acetic and uronic esters and results in swelling of cellulose which reduces its degree of crystallinity. Advantage of this pretreatment is that it can be employed at lower temperature and pressure as compared to other technologies. The liberation of 60 % lignin and 80% hemicellulose using 1.5% NaOH at 20 °C for 144 hours in wheat straw [1]. 44.3% yield of bioethanol is observed by grinding the banana peels and treating them with sodium hydroxide solution [10].

(c) Organosolv pretreatment:

This method of pretreatment involves the use of organic solvent which results in isolation of lignin. This technique is a pulping process utilized for eliminating lignin from lignocellulosic materials. Types of organic solvent utilized in this process are peracetic acid, ethanol, methanol, per formic acid, etc. The combination of organic solvents or water mixture generally used are 90% formic acid i.e., 50% water or alcohol mixture with 50% carbon dioxide at high pressure. Another combination is ammonia with ionic liquid which results in conversion of cellulose to glucose upto 97% for rice straw as feedstock [1].

(d) Ozonolysis:

Ozonolysis is potentially an effective method for pretreating the lignocellulosic biomass by degrading lignin and hemicellulose, to improve the production of fermentable sugars via enzymatic hydrolysis [12]. However cellulose is hardly affected. The ozonolysis can be performed for 2 hours with less than 60 mesh sized particles for achieving maximum oxidation of lignin [12]. This process decreases the lignin from 29% to 8%,

increasing the enzymatic hydrolysis yield from 0% to 57% [11]. Also the rate of enzymatic hydrolysis can be increased by a factor of 5. This pre-treatment has various advantages like effective lignin removal, at downstream processes toxic residues are not formed and room temperature and pressure are favorable for the reactions to be carried out. However, the process becomes expensive as a large quantity of ozone is required.

(e) Oxidative delignification:

Lignin biodegradation can take place using enzyme peroxidase with hydrogen peroxide as a catalyst. The cane bagasse can be pre-treated with hydrogen peroxide, enhancing its susceptibility to enzymatic hydrolysis. 2% H₂O₂ at 30°C within 8 h solubilized around 50% lignin and most of hemicellulose, and subsequently saccharifying cellulase at 45°C for 24 h can achieve 95% efficiency of glucose production [11]. Using wet oxidation and alkaline hydrolysis of wheat straw (20 g straw/l, 170 C, 5–10 min), can achieve 85% conversion yield of cellulose to glucose [11].

(f) Wet oxidation

In this pre-treatment, the lignocellulosic material is treated with high pressure air and water at elevated temperature generally above 120°C [13]. Typical pressure of air or oxygen falls in the range of 120 Psi to 480 Psi [13]. This pre-treatment effectively provides high susceptibility for enzymatic hydrolysis by fractionation of lignocellulosic material into cellulose rich solid fraction and hemicellulose fraction. Pre-treatment combination of alkali and wet oxidation improves the rate of oxidation of lignin and also the formation of furfural and hydroxymethylfurfural is prevented. Due to solubilization of hemicellulose components, acid formation takes place during initial reactions which catalyze the succeeding hydrolytic reactions by which lower molecular weight fragments, soluble in water are formed breaking down the hemicellulose. Compounds like phenol and carbon-carbon linkage under wet oxidation is very reactive due to which at higher temperatures lignin degradation is significant. Carbon dioxide, water and carboxylic acid are formed by decomposition of lignin. The alkaline wet oxidation of lignocellulosic material takes place at oxygen of 10 bar pressure and different temperatures [13]. At 170°C within 10 minutes about 65 % and 50% of delignification and solubilization of hemicellulose occurs respectively. The solid residue left over shows highest enzymatic convertibility of 85 (% w/w) of cellulose to glucose. On solubilization of hemicellulose, slight effect on oxygen

pressure and alkaline (sodium carbonate) addition is indicated [13]. Almost 96% hemicellulose solubilizes just with oxygen pressure and without carbonate [13]. Alkaline addition, decreases the formation of furfural regardless of presence or absence of oxygen by more than 10-fold. On other hand, oxygen presence significantly affects removal of lignin [13]. For untreated rice husk there is 42.2% by weight cellulose which is increased to 68.6% for pre-treated rice husk also the content of lignin reduces to 19.4% in untreated rice husk to as low as 0.82% in pre-treated rice husk [18]. Recovery of total cellulose variably ranges from 78% to 92% [18].

V. BIOLOGICAL PRETREATMENT:

The biological pretreatment incorporates the utilization of microorganisms like soft rot fungi, brown rot fungi and white rot fungi for delignification of lignocellulosic biomass and liberation of cellulose. White rot fungi are more efficient in degrading hemicellulose and lignin while the brown rot fungi are effective in degrading cellulose. The soft rot fungi also degrade both hemicellulose and lignin. The biological pretreatment induces structural loosening of cells with a simultaneous increase in porosity. The benefit of this pretreatment includes no requirement of chemicals, and less energy consumption due to the limiting use of mechanical equipments. Also it is proved to be environmental friendly and safe pre-treatment for removal of lignin from lignocellulosic material but yet a sufficiently rapid and controllable system has not been found, and the low rate of hydrolysis, low yields and long residence time obstructs the use of this pretreatment. Delignification upto 92% and reduction in cellulose upto 55.2% of sugarcane waste using *Aspergillus terreus* can take place [1]. Conversion of 35% of wheat straw to reducing sugars results in five weeks using 19 white-rot fungi [13]. *Aspergillus Niger* and *Aspergillus awamori* used for biological pre-treatment shows high yield of total sugars and bioethanol after fermentation [13].

VI. CONCLUSION

Bioethanol can be a potential biofuel that can replace gasoline, diesel etc., in order to conserve the exhausting oil reserves. But to increase the yield of bioethanol from lignocellulosic feedstock pretreatment is a crucial step that can make bioethanol compatible for commercial scale. The pre-treatment technology increases accessibility of enzyme to biomass and fermentable sugars

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yield [20]. This infers that an efficient and effective pretreatment technology suitable to the feedstock can reduce energy consumption and capital and operating costs of bioethanol production plants.

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