Intl J Engg Sci Adv Research 2015 Sep;1(3):160-167

Microbial Treatment of Cellulosic Waste: A Review

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***Abstract*— This review focused on the use of alternative energy technology due to increase in cost of crude oil, security concern regarding oil supply, and also because of some environmental issues such as global warming and air pollution. It is expected that requirement for alternative sources of bioenergy increases sharply in the future. Cellulosic waste produced in huge amount throughout the year worldwide and hence one of the most profusely available biomass existing on our planet is "Cellulosic Biomass" which is also an important "renewable biomaterial" for our society. The microbial treatment/bioconversion of cellulosic wastes results in the production of low cost and valuable products at the end. Bacteria and fungi are the microorganisms which are capable of degrading lignocellulolytic materials produce a complex of sequentially acting cellulolytic enzymes (cellulase), play crucial role in natural biodegradation process. Sugar yields have been shown to improve higher than 90% theoretical yield for biomass such as corn, grasses, and wood after pretreatment. The Pretreatment process split cellulose from polymers matrix and thus make cellulose more accessible for enzymatic hydrolysis.**

Keywords*— Cellulose; Cellulase; Lignocellulosic biomass; Pretreatment; Ethanol.

I. INTRODUCTION

Conversion of cellulosic biomass biotechnologically is potentially sustainable approach to novel bioprocess and products development. Cellulases composed of functionally and structurally discrete units called domains. These domains are folded independently and makes cellulases module [1]. Cellulose, linear long chain polysaccharide of glucose residues linked by β-1, 4-glycosidic linkages. Availability of cellulose in abundance makes it fascinating raw material for the production of important commodity products. But unfortunately most of the cellulosic waste is disposed of by biomass burning and is not limited to developing counties alone instead considered a global phenomenon. Cellulose can be breakdown to glucose with the help of cellulolytic bacteria. This biological conversion of cellulose to glucose is a much cheaper and favourable process [2]. Glucose is a product which finds wide application. For developed and developing countries municipal wastes have become a major problem during last decades. Municipal wastes have become a severe problem in developed and developing countries during last century [3]. An Indian city produces about 0.8 to 1 kg solid wastes per capita per day (waste management at military station, 2009). These wastes are collected and dumped into the landfills, causing major pollution [4,5,6]. This results in loss of potentially valuable materials that can be processed as fuel, fodder and fertilizer [7].

The biological treatment of these wastes appears to be most cost effective and carry a less negative environmental impact [8]. This process of biological treatment of wastes is also known as Composting. It is a self-heating, aerobic solid phase biodegradative process of organic materials under controlled conditions, which distinguishes it from natural rotting. The exploitation of the metabolic versatility of microorganisms is advantageous in biological waste treatment but the actual number of degraders of a target compound in a mixed culture may only represent 5-10% of the microbial community [9]. To understand how microorganisms may be manipulated and exploited to reduce the frequency of such breakdowns and shorten start-up times of biological waste treatment, the important bacterial strains actively involved in the degradation of food waste were isolated and screened [10].

Biological degradation of wastes during compositing affected by various types of microorganisms. It has been reported that even fungi are the most important microorganism which produce cellulase, there are some species of actinomycetes and bacteria that can also produce cellulases[11,12] and these are also involved in the process of degradation [13].The bioconversion of cellulosic materials is now a subject of intensive research as a contribution to the development of large scale conversion process beneficial to mankind [14].

A. Historical prospect of cellulose

For many years cellulose has been widely used material in various practical applications. The chemical composition, morphology and structure of cellulose were also remain unknown for many time. In 1837, Anselme Payen identified cellulose from plants chemically, then only the modern history of cellulose chemistry began actually. After that the establishment of cellulose physical and chemical structures has undergone innumerable period of struggle. Many scientists until the early 1920s believed that cellulose composed of few small molecules of cellobiose or glucose. The premiss that cellulose was a polymer accepted by only small number of scientists. For over ten years the controversial argument were remain continued. There were sufficient experimental data that finally provided proof that cellulose is a high molecular weight, covalently linked macromolecule [15]. In 1926 Sponsler and Dore, were the first to gave the idea that

cellulose is built up of long parallel chains comprising glycopyranose units and reconciling the idea of long molecules with the relatively short spacing [16].

B. Cellulose structure and its reactivity

Cellulose is the most abundant biomass on Earth [17]. It is the primary product of photosynthesis in terrestrial environments and the most abundant renewable bioresource produced in the biosphere [18,19].

Cellulose I, native cellulose molecules, found in fibril form is well known and its molecular architecture also have a higher degree of individuality. The high degree of individuality depends on its source (plant type or cell wall layer) [20]. Other crystal forms in which cellulose may occur are celluloses II, III, AND IV. Cellulose II structure can be formed from cellulose I by treatment with an aqueous solution of sodium hydroxide. To form cellulose II, cellulose I form undergoes a parallel chain arrangement of cellulose. This modification makes cellulose II more accessible to chemical treatment and hence more reactive. Fringe fibrillar model, describe the microfibrillar structure of cellulose polymer. This structure explained the partial crystallinity and reactivity of cellulose in relation to its microfibrillar structure [21].

In higher plants, cellulose microfibrils having diameter of 2-10 nm, are the main visual structural features of cellulose and cross-linked cross-linked by other cell wall components such as xyloglucans. Microcrystalline cellulose has been shown to be made up of two different crystal phases: Iα and Iβ[22,20]. The rate of diffusion of reactants is likely to be affected by these crystal structure and thus play a vital role in reactivity and accessibility of cellulose [23].

It has been reported that cellulose chains are so tightly packed (a significant feature of the highly ordered regions) that even small molecules such as water cannot enter these highly organized structural entities [24]. The restricted accessibility to these regions leads to modification of their reactivity to swelling and reactive agents such as cellulases. It is apparent that with this type of structure only the cellulose molecules situated on the surface of these aggregations would be vulnerable to the degrading actions of enzymes [23]. The accessible surface area is a potential determinant of the maximum rate of hydrolysis that can be attained only if the hydrolysis of cellulose occurs on the surface of the cellulose aggregations. It has been proposed that limited accessibility to cellulases results from firmly packed cellulose regions, as it serve as an important factor that contribute to the resistance of cellulose to degradation [25,26]. The term ‘amorphogenesis’ coined by Coughlan in 1985. He used this term to recommend a probable mechanism by which the swelling, delamination or diespersion of cellulosic substrate occurred, resulting in a decrease in the degree of fibrillar crystallinity and/or aggregation, and the formation of a better accessible surface by escalating the reactive internal surface. Therefore, amorphogenesis enhances the reactivity of the fibrous cellulosic substrates by raising the amount of cellulose directly accessible to the enzymes [27].

Chemicall reactivity of cellulose is governed by the sensitivity of the β-l,4-glycosidic bond and the presence of three hydroxyl groups on each repeating unit. The hydroxyl group present at sixth position functions as primary alcohol whereas hydroxyl group present at the second and third position function as secondary alcohol. In general, the relative reactivity of the hydroxyl groups can be expressed as OH-C6>>OH-C2>OH-C3 [28]. Depolymerisation of cellulose particularly induces by the cleavage of glycosidic bond, as glycosidic bond is sensitive to acid hydrolysis. The degree to which the depolymerisation of cellulose occurs by acid hydrolysis generally depends on temperature of the reaction, duration of the reaction, concentration of acid and also on acid strength.

C. Microbial Cellulases

Microbial cellulases have become the vital biocatalysts due to their wide spread industrial applications and complex nature. There is a wide diversity of microorganisms which include both bacteria and fungi, synthesized cellulases (an inducibile enzymes) during their growth on cellulosic materials.

Fungi having cellulolytic ability include Soft rot fungi (*Aspergillus niger; A. oryzae; A. terreus; Fusarium solani; Humicola insolens; Melanocarpus albomyces; Penicillium brasilianum; Trichoderma reesei; T. harzianum; Chaetomium cellulyticum; C. thermophilum; Neurospora crassa; P. fumigosum; Thermoascus aurantiacus;Mucor circinelloides; Trichoderma atroviride), Brown rot fungi(Coniophora puteana; Tyromyces palustris; Poria placenta; Lanzites trabeum; Fomitopsis sp.), White rot fungi (Phanerochaete chrysosporium; Sporotrichum thermophile; Trametes). Aerobic bacteria (Acinetobacter junii; Paenibacillus curdlanolyticus; Acidothermus cellulolyticus; Anoxybacillus sp.; Bacillus subtilis; B. amyloliquefaciens; B. licheniformis; B. circulan; B. flexus; Bacteriodes sp.; Cellulomonas biazotea; Cellvibrio gilvus; Eubacterium cellulosolvens; Geobacillus sp.; Microbispora bispora; Pseudomonas cellulosa; Salinivibrio sp.; Rhodothermus marinus) as well as anerobic bacteria(Acetivibrio cellulolyticus; Butyrivibrio fibrisolvens; Clostridium thermocellum; C. cellulolyticum; C. acetobutylium; C. papyrosolvens; Fibrobacter succinogenes; Ruminococcus albus) reported to have cellulolytic activity. It is also reported that there are certain species of Actinomycetes (Cellulomonas fimi; C. bioazotea; Streptomyces drozdowiczii; S. lividans; Thermomonospora fusca*;) have cellulolytic activity [29, 30].

These microorganisms can be anaerobic, aerobic, mesophilic or thermophilic. Among them, the genera of Cellulomonas, Trichoderma, Clostridium, Thermomonospora, and Aspergillus are the most extensively studied cellulose producer [31,11,32,33].

II. CLASSIFICATION OF CELLULASE

Free or cell associated extracellular cellulases are produced by microorganisms, to hydrolyze and metabolize cellulose (insoluble). During the last three decades, the biochemical

analysis of cellulose systems has been widely reviewed from fungi and anaerobic and aerobic bacteria [34].

A. *Endoglucanases or Endo-1, 4-β-D-Glucan Glucanohydrolases (EC 3.2.1.4)*

Endo-1, 4-β-glucanase (EG) also called endoglucanase, erratically cleave intermolecular β-1, 4-glucosidic linkages within the cellulose chain. The endoglucanases are generally assayed by viscosity reductions in carboxymethyl cellulose (CMC) solution. exoglucanases and endoglucanases differ in theri mode of action. In case of endoglucanases, specific viscosity of CMC reduces significantly with minute hydrolysis due to intramolecular cleavages, whereas in case of exoglucanases, hydrolysis of long chain occur from the ends in a progressive process [35,19]. Endoglucanases cut arbitrary at internal amorphous sites in the cellulose polysaccharide chain and thus generating oligosaccharides of various lengths and therefore new chain ends. It is generally active against soluble derivatives of cellulose such as CMC, cellooligosaccharides and acid-swollen amorphous cellulose [36].

B. *Exoglucanase or 1, 4-β-D-Glucan Cellobiohydrolases (Cellobiohydrolases) (EC 3.2.1.91)*

Exoglucanases act on both the reducing or nonreducing ends of cellulose polysaccharide chains and liberating either glucose or cellobiose as key products. Exoglucanases can successfully work on microcrystalline cellulose, apparently peeling cellulose chains from the microcrystalline structure [35]. However, they are dormant against cellobiose or substituted soluble celluloses such as CMC [34]. Among insoluble cellulosic substrates, Avicel has been used for measuring exoglucanase activity. Unfortunately, soluble collodextrins and amorphous cellulose are substrates for both purified exoglucanases and endoglucanases. Consequently, unlike endoglucanases and β-glucosidases, exoglucanases have no specific substrates for it within the cellulase mixtures [37].

C. *β - Glucosidases or β-D-Glucoside Glucohydrolases (EC 3.2.1.21)*

β-Glucosidases (BG s) that do not contain a CBM hydrolyze soluble cellodextrins and cellobiose to glucose. β-D-glucosidases produce glucose in the aqueous phase by hydrolyzing soluble cellobiose and other cellodextrins with a degree of polymerization (DP) up to six. The hydrolysis rate decreases remarkably as the degree of polymerizations of substrate increases [1,19]. The activity of BG on insoluble cellulose is negligible. BGs degrade cellobiose, which is a known inhibitor of CBH and endoglucanase. Most of the time, aerobic fungi produce extracellular BGs, and anaerobic bacteria keep their BGs in cytoplasm. BGs have a pocket-shaped active site, which allows them to bind the nonreducing glucose unit and clip glucose off from cellobiose or cellodextrin [38]. It is inactive against crystalline or amorphous cellulose [34].

D. *Cellobiose Phosphorylase or Cellobiose: Orthophosphate Alfa–D-Glucosyl Transferase (EC 2.4.1.20)*

In cellulose degradation, cellobiose phosphorylase (CBP) plays a significant role by catalyzing the reversible phosphate-dependent hydrolysis of cellobiose (the major product of cellulose degradation) into α-D-glucose 1-phosphate and D-glucose [39]. It was first discovered by Ayers [40] in cells of Ruminococcus flavefacience.

Cellobiose + H3PO4 = alfa – D –glucose 1-P +glucose

III. SCREENING AND ISOLATION OF CELLULOSE DEGRADING BACTERIA

Culturable, cellulase producing bacteria have been isolated over the years from a wide variety of sources such as the feces of ruminants such as cows, organic matter, soil, decaying plant material from forestry or agricultural waste and composting heaps and extreme environments like hotsprings [41]. Cellulase producing bacteria can be screened by enrichment growth on microcrystalline cellulose as a sole source of carbon. Then determine the molecular community structure of the environment and analyze whether families containing cellulase producing species are present by the extraction of 16S rDNA/RNA. Potential cellulase producing can be isolated by subculturing from the enrichment culture on cellulose as a sole carbon source. This method was used to recognize cellulase producing bacteria in the deep subsurface of the Homstake gold mine, Lead, South Dakota, USA [42]. In microbial isolates, bacterial cellulase activity can be screened typically on carboxymethylcellulose (CMC) containing plates [43].

For a fast screening of cellulase producing bacteria, the agar medium containing 0.5% (W/V) carboxymethyl cellulose (CMC) as sole carbon source, after incubation, the plate was flooded with 1% (W/V) Congo red [44]. After 20 minutes, the dye is decanted and the plates are again flooded with 5M NaCl which is poured off after 20-30 minutes. Positive colonies can be detected, as it is surrounded by a pale orange to clear zone against red background (clear zone result from the cellulase producing activity of bacteria). Although cellulolytic bacteria can be screened directly on such plate, but for isolation of active colonies can be favoured by replica plating from master plate as flooded reagent impairing isolation [34]. With this method, zones of hydrolysis are not easily discernable and can be timely. Flooding plate with Gram's iodine in place of Congo red or hexadecyltrimethyl ammonium bromide, gave a faster and highly recognizable result, as recently reported by Kasana and his colleagues [45].

IV. WASTE UTILIZATION BY CELLULOLYTIC MICROORGANISMS

Cellulose is the major part of plant biomass. Consequently, the wastes produced from agricultural fields, forests, and agro industries contain a huge amount of unutilized or underutilized cellulose. Industrial and agricultural wastes are among the causes which contribute to environmental pollution [46]. These wastes generally gather in the environment causing

pollution problem [47]. Nowadays, these so called wastes are prudently converted into valuable products such as enzymes [48], Sugar [49], biofuels, cheap energy sources for fermentation, chemicals, improved animal feeds and human nutrients[50], which is accomplished by cellulase. Therefore, the unnecessary biomass and agrowastes are successfully utilized for the production of sugar, enzymes and alcohols [51,52,53,54,55].

V. ETHANOL PRODUCTION FROM LIGNOCELLULOSICS MATERIALS

Three main components of lignocelluloses are hemicelluloses, cellulose and lignin. Cellulose and hemicellulose consist of chains of sugar molecules and the typical compositions of the cellulose in common agricultural residues and wastes were as follow; Hardwood 24-40%, Softwood 25-35%, Nut shells 25-30%, Corn cobs 45%, Paper 85-99%, Cotton seeds hairs 80-95%, Newspaper 40-55%, Waste paper from chemical pulps 60-70%, Primary wastewater solids 8-15%, Fresh bagasse 33.4%, Swine waste 6%, Solid cattle manure 1.6-4.7%, Coastal Bermuda 25%, Switch grass 45%, S32 rye grass (early leaf) 21.3%, S32 rye grass (seed setting) 26.7% Orchard grass (medium maturity) 32%,Grasses (average values for grasses) 25-40% [56,58,59,60,61]. These chains of sugar molecules can be hydrolysed to produce monomeric sugars and the ordinary baker's yeast can then be used to ferment these monomeric sugars. There are various ways through which ethanol can be produced from lignocellulosic materials, but the main steps that all processes comprises are same and these main steps include the hydrolysis of the hemicellulose and the cellulose to monomer sugars, fermentation and product recovery and concentration by distillation. The major difference between the processes is the hydrolysis steps, which can be done by enzymatically, dilute acid, concentrated acid. Some of the process steps are more or less the same, independent of the hydrolysis method used [57].

VI. PRETREATMENT OF LIGNOCELLULOSIC MATERIALS

For a long time, the effect of pretreatment of lignocellulosic materials has been known [62]. Pretreatment of the lignocellulosic materials is crucial since hydrolysis of nonpretreated materials is slow, thus this results in low product yield. There are some pretreatment methods which increase the pore size and decrease the crystallinity of cellulose [63]. Availability of cellulose to cellulolytic enzymes can also be increased by pretreatment, this in turn decreases the requirement of enzyme and, thus, the ethanol production cost. The pretreatment of lignocellulosic materials, not only improve the biodigestibility of the wastes for ethanol production, but this will also results in the improvement of difficult biodegradable materials, and enhance the yield of ethanol from the cellulosic wastes [64].

Pretreatment must meet the certain prerequisites, which are as follow:

(1) advance the formation of sugars or the capacity to consequently form sugars by enzymatic hydrolysis;

(2) circumvent the loss or degradation of carbohydrate;

(3) circumvent the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and

(4) should be cost-effective. Physico- chemical, chemical, Physical, and biological processes have been used for pretreatment of lignocellulosic materials [31].

A. Physical Pretreatment

a) Mechanical comminution: A combination of grinding, milling and chipping can be used to comminute waste materials so as to reduce cellulose crystallinity. After chipping, the size of the materials is generally 10–30 mm. However, after grinding or milling, the size of materials is usually in the range 0.2–2 mm. In breaking down the cellulose crystallinity of spruce and aspen chips, vibratory ball milling has been found to be more efficient. It also takes part in improving the digestibility of the biomass than ordinary ball milling [65]. In mechanical comminution of agricultural materials, the power requirement depends on the waste biomass characteristics and the final particle size [66].

b) Pyrolysis: For pretreatment of lignocellulosic materials, pyrolysis has also been used. Cellulose rapidly decomposes to produce residual char and gaseous products, when the materials are treated at temperatures greater than 300 degree Celsius [67,68].

B. Physico-chemical pretreatment

a) Steam explosion (autohydrolysis): Steam explosion, the most commonly used method for pretreatment of lignocellulosic materials [62]. In this method, high-pressure saturated steam is used to treat chipped biomass and then the pressure is reduced rapidly, this makes the materials endure an explosive decompression. In Steam explosion, before the material is exposed to atmospheric pressure, it is usually commenced at a temperature of 160–260 degree celsius (corresponding pressure range 0.69 to 4.83 MPa) for several seconds to a few minutes. The process increasing the potential of cellulose hydrolysis by causing lignin transformation and hemicellulose degradation because of high temperature [31]. Steam explosion pretreatment is affected by some factors such as temperature, chip size, time and moisture content [69].

b) Ammonia fiber explosion (AFEX): In Ammonia fiber explosion (AFEX), lignocellulosic materials are exposed to liquid ammonia at high pressure and temperature for a period of time and then reduced the pressure quickly. The dosage of liquid ammonia, in a usual AFEX process is 1–2 kg ammonia/kg dry biomass, residence time 30 min and temperature 90 degree celsius [31].

c) Ammonia Recycle Percolation (ARP): In Ammonia Recycle Percolation (ARP) process, a packed bed reactor containing the biomass feed stock, through which aqueous ammonia of concentration between 5–15% (wt%), is sent at a rate of about 5mL/min. In contrast to AFEX process, moderately high temperatures (1400C to 2100C) and longer reactions times are seen in ARP process, creating higher energy costs [70]. The ability to eradicate a majority of the

lignin (75–85%) and solubilise more than half of the hemicellulose (50–60%) while maintaining high cellulose content are the advantages associated with this process over AFEX [71].

d) Supercritical fluid (SCF) pretreatment: In a state above critical pressure and critical temperature where liquids and gases can coexist, a material called supercritical fluid is used, which can be either gas liquid. Under standard conditions, it shows distinctive properties that are different from those of either liquids or gases, it possesses a liquid like density and reveals gas like transport properties of diffusivity and viscosity [72]. Consequently, Supercritical fluid (SCF) pretreatment process overcomes the mass transfer limitations which generally encountered in other pretreatments, as SCF has the capacity to penetrate the crystalline structure of lignocellulosic biomass. Further, the lower temperatures used in this Supercritical fluid (SCF) pretreatment process assists in the firmness of the sugars and avoids degradation examined in other pretreatments process. The consequence of pretreatment of wet and dry wheat straw by supercritical CO2 only and by a combination of both CO2 and steam under different operating conditions (residence time temperature in the reactors was investigated by Alinia and coworkers (2010) [73].

e) Liquid hot water (LHW): Liquid hot water (LHW) pretreatment process, greatly like the steam explosion process uses water at high temperatures and high pressures in order to maintain its liquid form and thus promote breakdown and separation of the lignocellulosic matrix. Temperatures can vary in the range from 160°C to 240°C over a period of time ranging from a few minutes up to an hour. The types of sugar formation are dominated by sugar formation whereas the amount of sugar formation dominated by time [74].

C. Chemical pretreatment

a) Alkaline pretreatment: Pretreatment of lignocellulosic biomass by alkaline pretreatment process involves the use of bases, such as sodium, calcium, potassium, and ammonium hydroxide. The use of an alkali results in the structural alteration of lignin, partial decrystallization of cellulose, cellulose swelling [75,76], and partial solvation of hemicelluloses by causing the degradation of ester and glycosidic side chains [77,78].

b) Acid pretreatment methods: In these acid pretreatment methods, concentrated and diluted acids are used to smash the firm structure of the lignocellulosic material. The most usually used acid is dilute sulphuric acid (H2SO4), which has been commercially used to pretreat a wide range of biomass types such as switchgrass[79,80], corn stover [81,82], spruce (softwood)[83], and poplar [84,85]. Acid pretreatment i.e., removal of hemicelluloses followed by alkali pretreatment i.e., removal of lignin results in reasonably pure cellulose. One of the main advantages associated with acid pretreatment is that in these processes a consequent enzymatic hydrolysis step is sometimes not needed because the acid itself hydrolyze the biomass so as to yield fermentable sugars [86].

c) Green Solvents: In the last decade, due to the tunability of the solvent chemistry and so the ability to dissolve a wide range of biomass types, the processing of lignocellulosic biomass with ionic liquids (IL) and other solvents has received importance. Ionic liquids are salts, which exist as liquids at room temperature and having very low vapor pressure, usually composed of a little anion and a large organic cation. The anion and cation chemistry can be tuned to produced a wide variety of liquids which can dissolve a number of biomass types—corn stover [87], cotton [88], bagasse [89], switchgrass [90], wheat straw[91], and woods of different hardness [92] (pine, poplar, eucalyptus, and oak). The IL having anion which forms hydrogen bonds with cellulose (sugar hydroxyl protons) in a 1:1 ratio and breaks up the cellulose crystalline hydrogen bonded structure, as a result making it more accessible and amorphous to enzymatic hydrolysis.

d) Wet Oxidation: In wet oxidation oxygen is utilize as an oxidizer for compounds dissolved in water. During this wet oxidation process, there occur following two reactions; i) one is a low temperature hydrolysis and, ii) second is a high temperature oxidation reaction [93].

D. Biological pretreatment methods

Microorganisms such as white-, brown and soft-rot fungi are used in biological pretreatment processes to degrade hemicelluloses and lignin in waste materials [94]. Both cellulose and lignin are attacked by white as well as soft rots fungi whereas as brown rots attack mainly cellulose. The most effective basidiomycetes for biological pretreatment of lignocellulosic materials are white-rots fungi [95]. Mild environment conditions and low energy requirementare the advantages associated with biological pretreatment. However, in most biological pretreatment processes, the rate of hydrolysis lignocellulosic materials is very low.

VII. ENZYMATIC HYDROLYSIS AND FERMENTATION

After pretreatment, cellulose is hydrolyzed with the help of cellulase. Cellulase production is common among fungi and this occur in wide range of species, this includes Trichoderma, Penicillum and Aspergillus. Cellulolytic bacteria as compared to fungi produce cellulolytically active enzymes in small amount.

There are different ways through which the process of enzymatic hydrolysis can be designed. Pretreatment process followed by some steps, including hydrolysis and fermentation, which can be run as separate hydrolysis and fermentation (SHF) or as simultaneous saccharification and fermentation (SSF). The ability to carry out each step under optimal conditions, i.e. enzymatic hydrolysis at 45–50°C and fermentation at about 30°C is one of the advantage associated with SHF. To run fermentation in continuous mode with cell recycling is also possible. In SHF, one of the major drawbacks

is that sugars released inhibit the enzymes during hydrolysis. In SSF, fermenting microorganism e.g. Saccharomyces cerevisiae (ordinary baker's yeast), immediately consumed the glucose produced. This fermenting microorganism avoids end-product inhibition of Beta-glucosidase. The ethanol produced by fermentation process can also act as an inhibitor in hydrolysis but not as strongly as glucose or cellobiose. Another important advantage of SSF as compared with SHF is that that when hydrolysis and fermentation are performed in one reactor process integration can be obtained which in turn reduces the number of reactors needed. In SSF, the temperature of about 35°C is a compromise but the development of thermotolerant yeast strains is expected to enhance the performance of SSF. A major disadvantage of SSF is the difficulty in recycling and reusing the yeast since it will be mixed with the lignin residue. An often-claimed advantage is a reduction in the sensitivity to infection in SSF [96,97,98].

The overall the ethanol production and ethanol yield rate depend not only on the yield of sugar but also on the fermentability of the solution. This is influenced by the concentration of the soluble substances present in the original raw material, released during pretreatment, or formed in the pretreatment step. The concentrations of these substances in the fermentation step depend on the configuration of the preceding process steps [57].

The most frequently used microorganism for fermenting ethanol in industrial processes is S. cerevisiae, which has proved to be very robust and well suited to the fermentation of lignocellulosic hydrolysates [99]. Zymomonas mobilis can ferment glucose to ethanol with higher yields, due to the production of less biomass, but is less robust[100,101].

VIII. CONCLUSION

Lignocellulolytic microorganisms, have attracted a great deal of interest as biomass degraders for largescale applications due to their ability to produce large amounts of extracellular lignocellulolytic enzymes. Cellulosic biomass utilization becomes a subject of interest worldwide in view of rapid depletion of our oil reserves. Using lignocellulosic materials such as agriculture residues, gasses, forestry wastes and other low cost biomass can significantly reduce the cost of raw materials as compared to corn for ethanol production. Pretreatment of lignocellulosic material is required to enhance accessible surface area to decrystallize cellulose and to remove hemicelluloses and lignin.

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