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Utilization of hemicellulosic fraction of lignocellulosic biomaterial for bioethanol production

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ABSTRACT

Bioethanol, a renewable fuel, is becoming increasingly important as a consequence of greater concern for the increasing greenhouse effect, depleting oil reserves, and rising oil prices. In today's need of production of bioethanol from lignocellulosic biomass requires attention towards utilization of hemicellulosic fraction to convert xylose, the second most abundant sugar and major monomer carbohydrate present in the hemicellulosic fraction of lignocellulosic biomaterial, to ethanol along with the glucose which is comparatively easier to ferment by the microorganisms. The present review paper mainly focuses on the availability of hemicellulosic content in various lignocellulosic biomaterials, its pretreatment/hydrolysis methods, microorganisms, and fermentation parameters. Recent trends, major challenges and perspective of future development are highlighted.

Keywords: Hemicellulose, pretreatment, ethanol, xylose utilizing microorganisms.

INTRODUCTION

The increased demand for crude oil, manifested in trading prices, $80/barrel in 2006 to $140/barrel in 2008, has renewed the interest in exploiting lignocellulosic feedstock not only for liquid transportation fuel but also for the production of chemicals and materials of industrial importance, i.e., the development of carbohydrate-based biorefineries [1-3]. For the Global ethanol market, Brazil has more than 300 plants producing 15 billion liters per year and supplying 3 million cars with pure ethanol. In the US, there are more than 80 plants producing 10 billion liters per year. Whole of Europe (eastern and western) produces 4.5 billion litres per year; China produces 3 billion liters of ethanol per year, while India produces only 2.7 billion liters of ethanol annually. This has led greater focus on research and development aimed at sustainable production of fuels and chemicals from renewable lignocellulosic feedstocks from agriculture and forestry. Such feedstocks are composed of cellulose, hemicellulose, and lignin. The chemical association of these polymers is shown in Figure 1. Cellulose is a homopolymer of glucose, while hemicellulose is heteropolymer composed of the hexose sugars e.g. glucose, mannose, and galactose, and the pentose sugars e.g. xylose and arabinose. The relative proportion of the

individual sugars depends on the raw material; the hemicellulose fraction of hardwoods and agricultural raw materials is rich in pentose sugars, while softwood hemicellulose only contains minor fractions of the pentose sugar D-xylose [4]. The cellulose, hemicellulose, and lignin contents in common agricultural residues and wastes are given in Table 1 [5].

Complete substrate utilization is one of the prerequisites to render lignocellulosic ethanol processes economically favourable [6]. This means that all types of sugars in cellulose and hemicellulose must be converted to ethanol. Processes capable of efficiently converting the soluble carbohydrates in hemicelluloses hydrolysates to ethanol are necessary to achieve high overall biomass-to-ethanol process yield. In this article a brief review on utilization of hemicelluloses as a raw material for ethanol production, is presented.

Production Scheme

The biochemical production of ethanol from hemicellulosic portion of lignocellulosic biomass involves conditioning the residues by preliminary treatment, hydrolysis of hemicellulosic components to sugars and further converting them to alcohol that must then be concentrated for use as fuels or chemical reagents.

Pretreatment

The pretreatment of lignocellulosic biomass and its hydrolysis is vital before fermentative conversion to ethanol. Various pretreatment options are available now to fractionate, solubilize, hydrolyze and separate cellulose, hemicellulose, and lignin components. These include physical, physicochemical, chemical and biological pretreatment.

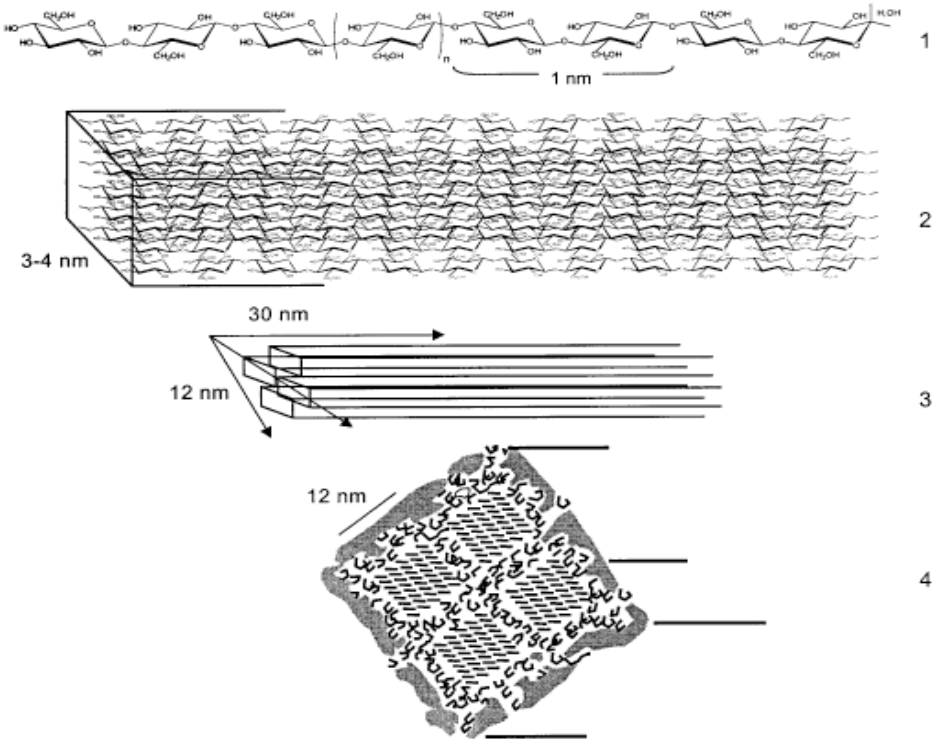

Figure 1: Chemical association in lignocellulosic material: (1) the cellulose backbone with length of its basic unit, cellobiose; (2) elementary fibril containing cellulose chains; (3) crystalline cellulose; (4) cross section of microfibril, showing strands of cellulose molecules embedded in a matrix of hemicellulose and lignin.

Physical Pretreatment

Mechanical comminution

Feedstocks can be comminuted by an arrangement of chipping, grinding and milling to reduce cellulose crystallinity. The size of the materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding. Vibratory ball milling has been found to be more of use in breaking down the cellulose crystallinity of spruce and aspen chips and getting better digestibility of the biomass than normal ball milling [7]. The power requirement of mechanical comminution of agricultural materials depends on the required final particle size and the biomass characteristics.

Table 1: The contents of cellulose, hemicellulose, and lignin in common lignocellulosic materials

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
Wheat straw	30	50	20
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seed hairs	85-95	5-20	0
Newspaper	40-55	25-40	18-30
Waste papers from chemical pulps	60-70	10-20	5-10
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0
Water-hyacinth	18.4	49.2	3.55

Pyrolysis

Pyrolysis has also been used for pretreatment of lignocellulosic materials, at temperatures greater than 300˚C, cellulose rapidly decomposes to produce gaseous products and residual char [8, 9]. The decomposition is much slower and less volatile products are formed at lower temperatures. Mild acid hydrolysis (1 N H_2SO_4, 97˚C, 2.5 h) of the residues from pyrolysis pretreatment has resulted in 80-85% conversion of cellulose to reducing sugars with more than 50% glucose. The process can be enhanced with the presence of oxygen [9]. When zinc chloride or sodium carbonate is added as a catalyst, the decomposition of pure cellulose can occur at a lower temperature.

Physico-chemical Pretreatment

Steam explosion (autohydrolysis)

Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials [10]. 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, a 90% efficiency of enzymatic hydrolysis has been achieved in 24 h for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips. The factors that affect steam explosion pretreatment are residence time, temperature, chip size and moisture content [11]. Optimal hemicellulose solubilization and hydrolysis can be achieved by either high temperature and short residence time (270˚C, 1 min) or lower temperature and

longer residence time: 190˚C, 10 min; Duff and Murray 1996 [11] and 170°C for 60 min; Lee, Shi, Venditti et al 2009 [12].

Addition of H_2SO_4 (or SO_2) or CO_2 in steam explosion can effectively improve enzymatic hydrolysis, decrease the production of inhibitory compounds, and lead to more complete extraction of hemicellulose [13]. 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. Steam explosion is recognized as one of the most cost-effective pretreatment processes for hardwoods and agricultural residues, but it is less effective for softwoods [14].

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 fermentation processes [15]. Because of the formation of degradation products that are inhibitory to microbial growth, enzymatic hydrolysis, and fermentation, pretreated biomass needs to be washed by water to remove the inhibitory materials along with water-soluble hemicellulose [16]. The water wash decreases the overall saccharification yields due to the removal of soluble sugars generated due to hydrolysis of hemicellulose. Typically, 20–25% of the initial dry matter is removed by water wash [17].

Ammonia fiber explosion (AFEX)

AFEX is another type of physico-chemical pretreatment in which lignocellulosic materials are exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is suddenly reduced. The concept of AFEX is similar to steam explosion. In a typical AFEX process, the dosage of liquid ammonia is 1-2 kg ammonia/kg dry biomass, temperature 90˚C, and residence time 30 min. AFEX pretreatment can significantly improve the saccharification rates of various herbaceous crops and grasses. The AFEX pretreatment does not significantly solubilize hemicellulose compared to acid pretreatment (discussed in the following section) and acid-catalyzed steam explosion. Mes-Hartree, Dale and Craig 1988 [17] compared the steam and ammonia pretreatment for enzymatic hydrolysis of aspen wood, wheat straw, wheat chaff, and alfalfa stem and they found that steam explosion solubilizes the hemicellulose, while AFEX did not. The composition of the materials after AFEX pretreatment was essentially the same as the original materials. Over 90% hydrolysis of cellulose and hemicellulose has been obtained after AFEX pretreatment of Bermuda grass (approximately 5% lignin) and bagasse (15% lignin). However, the AFEX process was not very effective for the biomass with high lignin content such as newspaper (18–30% lignin) and aspen chips (25% lignin). Hydrolysis yield of AFEX-pretreated newspaper and aspen chips was reported to be 40% and below 50%, respectively [16].

To reduce the cost and protect the environment, ammonia must be recycled after the pretreatment. In an ammonia recovery process, superheated ammonia (200˚C) was used to vaporize and strip the residual ammonia in the pretreated biomass and the evaporated ammonia was then withdrawn from the system by a pressure controller for recovery. The ammonia pretreatment does not produce inhibitors for the downstream biological processes, so water wash is not necessary [17, 18]. It seems particle size does not play any significant role in AFEX pretreatment.

CO_2 explosion

Similar to steam and ammonia explosion pretreatment, CO_2 explosion is also used for pretreatment of lignocellulosic materials. It was hypothesized that CO_2 would form carbonic acid and increase the hydrolysis rate. Dale and Moreira 1982 used this method for pretreatment of alfalfa (4 kg CO_2/kg fibre at the pressure of 5.62 MPa) and obtained 75% of the theoretical glucose during 24 h of the enzymatic hydrolysis [19]. The yields were relatively low compared to steam or ammonia explosion pretreatment, but high compared to the enzymatic hydrolysis without pretreatment. Zheng, Lin and Tsao 1998, compared CO_2 explosion with steam and ammonia explosion for pretreatment of recycled paper mix, sugarcane bagasse, and repulping waste of recycled paper, and found that CO_2 explosion was more cost-effective than ammonia explosion and did not cause the formation of inhibitory compounds that could occur in steam explosion [20].

Chemical Pretreatment

Acid hydrolysis

Concentrated acids such as H_2SO_4 and HCl have been used to treat lignocellulosic materials [21]. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible [22]. Dilute acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. The dilute sulphuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis. At moderate temperature, direct saccharification suffered from low yields because of sugar decomposition. High temperature in dilute acid treatment is favourable for cellulose hydrolysis [16]. Lee, Rodrigues and Jeffries 2009, have reported 0.032g/g oxalic acid concentration at 168°C for 74 min as the optimum value for ethanol production from corncob [23]. Recently developed dilute acid hydrolysis processes use less severe conditions and achieve high xylan to xylose conversion yields and is necessary to achieve favourable overall process economics because xylan accounts for up to one third of the total carbohydrate in many lignocellulosic materials [24].

There are primarily two types of dilute acid pretreatment processes: high temperature (more than 160˚C), continuous-flow process for low solids loading (5–10% weight of substrate/weight of reaction mixture) [25, 26], and low temperature (less than 160˚C), batch process for high solids loading (10–40%) [27]. Cara, Ruiz, Oliva et al 2008, reported as high as 83% hemicellulosic sugars recovery from the olive tree biomass by using 1% H_2SO_4 at 170˚C treatment [28]. Although dilute acid pretreatment can significantly improve the cellulose hydrolysis but its cost is usually higher than some physico-chemical pretreatment processes such as steam explosion or AFEX. A neutralization of pH is necessary for further enzymatic hydrolysis or fermentation processes. Utilization of hemicellulose hydrolysate of various lignocellulosic materials for production of ethanol using dilute acid treatment is given in Table 2.

Table 2: Production of ethanol from hemicellulose hydrolysate using dilute acid treatment

Source of hydrolysate	Microorganism	$Y_{p/x}$ (g/g)	$Q_p$max (g/L.h)	Reference
Wheat straw	*P. stipitis*	0.24	0.03	[29]
Sugar cane bagasse	*P. stipitis* CBS 5773	0.35	0.48	[30]
Sugar cane bagasse	*P. stipitis* CBS 7126	0.37	0.57	[31]
Red oak	*P. stipitis* CBS 5773	0.46	-	[32]
Red oak	*P. tannophilus* NRRL 2460	0.25	-	[33]
Wheat straw	*P. stipitis* NRRL 7154	0.35	0.30	[34]
Water-hyacinth	*Pichia stipitis* NRRL Y-7124	0.35	0.18	[35]
Water-hyacinth	*Pichia stipitis* NCIM-3497	0.425	0.176	[36]

Alkaline hydrolysis

Some bases can also be used for pretreatment of lignocellulosic materials and the effect of alkaline pretreatment depends on the lignin content of the materials [16]. The mechanism of alkaline hydrolysis is based on saponification of intermolecular ester bonds cross-linking xylan hemicelluloses and other components. The porosity of the lignocellulosic materials increases with the removal of the cross linkages. Dilute NaOH treatment of lignocellulosic materials caused swelling; leading to an increase in internal surface area, decrease in the degree of polymerization, decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure.

The digestibility of NaOH treated hardwood increased from 14% to 55% with the decrease of lignin content from 24–55% to 20%. However, poor effect of dilute NaOH pretreatment was observed for softwoods with lignin content greater than 26% [7]. Dilute NaOH pretreatment was also effective for the hydrolysis of straws with relatively low lignin content of 10–18%. Chosdu, Hilmy, Erizal et al 1993 used the combination of irradiation and 2% NaOH for pretreatment of corn stalk, cassava bark and peanut husk [37]. The glucose yield of corn stalk was 20% in untreated samples compared to 43% after treatment with electron beam irradiation at a dose of 500 kGy and 2% NaOH, but the glucose yields of cassava bark and peanut husk were only 3.5% and 2.5%, respectively. Ammonia was also used for the pretreatment to remove lignin. Iyer, Wu, Kim et al 1996 described an ammonia recycled percolation process (temperature, 170˚C; ammonia concentration, 2.5–20%; reaction time, 1 h) for the pretreatment of corn cobs/stover mixture and switch grass [38]. The efficiency of delignification was 60–80% for corn cobs and 65–85% for switch grass.

Oxidative delignification

Lignin biodegradation could be catalyzed by the peroxidase enzyme in the presence of H_2O_2 [39]. The pretreatment of cane bagasse with hydrogen peroxide greatly enhanced its susceptibility to enzymatic hydrolysis. About 50% lignin and most hemicellulose were solubilized by 2% H_2O_2 at 30˚C within 8 h, and 95% efficiency of glucose production from cellulose was achieved in the subsequent saccharification by cellulase at 45˚C for 24 h [39]. Bjerre, Olesen and Fernqvist 1996 used wet oxidation i.e. alkaline hydrolysis of wheat straw (20 g straw/L, 170˚C, 5-10 min), and achieved 85% conversion yield of cellulose to glucose [40].

Organosolv process

In the organosolv process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4) is used to break the internal lignin and hemicellulose bonds. The organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol [41]. 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 not necessary for satisfactory delignification [42]. Usually, a high yield of xylose can be obtained with 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 or fermentation.

Biological Pretreatment

In biological pretreatment processes, microorganisms such as brown-, white- and soft-rot fungi are used to degrade lignin and hydrolyse hemicellulose in waste materials [43]. Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective *Basidiomycetes* for biological pretreatment of lignocellulosic

materials. Hatakka 1983 studied the pretreatment of wheat straw using 19 varieties of white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in five weeks [44]. Similar conversion was obtained in the pretreatment by *Phanerochaete sordida* 37 and *Pycnoporus cinnabarinus* 115 in four weeks. In order to prevent the loss of cellulose, a cellulase-less mutant of *Sporotrichum pulverulentum* was developed for the degradation of lignin in wood chips. Akin, Rigsby, Sethuraman et al 1995 also reported the delignification of Bermuda grass by white-rot fungi [45]. The biodegradation of Bermuda grass stems was improved by 29–32% using *Ceriporiopsis subvermispora* and 63–77% using *Cyathus stercoreus* in 6 weeks.

The white-rot fungus *P. chrysosporium* produces lignin-degrading enzymes, lignin peroxidases and manganese-dependent peroxidases, during secondary metabolism in response to carbon or nitrogen limitation [46]. Both enzymes have been found in the extracellular filtrates of many white-rot fungi for the degradation of wood cell walls. Other enzymes including polyphenol oxidases, laccases, H_2O_2 producing enzymes and quinone-reducing enzymes can also degrade lignin [47]. The advantages of biological pretreatment include low energy requirement and mild environmental conditions. However, the rate of hydrolysis in most biological pretreatment processes is very low.

Hydrolysate Composition and Detoxification

Hemicellulose hydrolysates typically contain monomeric sugars other than D-xylose, such as D-glucose, D-mannose, D-galactose and L-arabinose [48, 49]. In addition hydrolysates contain appreciable amount of oligosaccharides as a result of incomplete hydrolysis of hemicellulose polysaccharides. Often, a secondary dilute acid hydrolysis step is used after primary pretreatment to hydrolyse oligomeric sugars into monomeric sugars before fermentation [50, 51]. In addition to mixed sugars and oligosaccharides, inhibitory component are usually present in pretreated materials [52]. Such compounds arise from hydrolytic release of compounds present in unretracted biomass (e.g., organic acids, extractives, and phenolics), reaction of carbohydrates and other solubilized components to form degradation products (e.g., furfural and hydroxymethyl furfural), and corrosion resulting in the release of inorganic ions [52]. The amounts of inhibitors produced depend greatly on process conditions and configuration. To be of use in a practical process, a microorganism must remain metabolically active in the presence of inhibitory compounds generated during pretreatment with, at most, relatively low-cost detoxification measures taken. Various methods for detoxification of the hydrolysates have been developed [53]. These include treatment with ion-exchange resins, charcoal or the ligninolytic enzyme laccase, pre-fermentation with the filamentous fungus *Trichoderma reesei*, removal of non-volatile compounds, extraction with ether or ethyl acetate, and treatment with alkali (lime) or sulfite. Persson, Larsson, Jonsson et al 2002 employed counter current flow supercritical fluid extraction to detoxify a dilute acid hydrolysate of spruce prior to ethanol fermentation with Baker's yeast [54]. Hemicellulose acid hydrolysate was heated to 100°C, held at that temperature for 15 min to remove or reduce the concentration of volatile components. Any loss in volume during boiling was replaced with heated distilled water. Hydrolysate was then over limed with solid $Ca(OH)_2$ up to pH 10.0, in combination with 0.1% sodium sulfite, filtered to remove insolubles and then reacidified to pH 6.0±0.2, with 1 N sulfuric acid. The filtrate was concentrated under vacuum at 25 °C to achieve (5–6% w/v) of xylose concentration.

Selection of Microorganism

A variety of yeast, fungi and bacteria are capable of fermenting xylose to ethanol and numerous reviews of xylose fermentation are available [55, 56]. Only a few of the known xylose

fermenting microorganisms are generally considered promising for carrying out direct high yield fermentation of xylose to ethanol.

Yeasts

Fein, Tallim and Lawford 1984 isolated 7 strains, which were capable of fermenting xylose to produce ethanol from crude wood hydrolysate in batch culture [57]. Xylitol was found to be one of the major by-products and the amount of xylitol varied with the strain used. Among yeast strains such as *Candida tropicalis*, *Candida shehatae* and *Pachysolen tannophilus*, strains of *Pichia stipitis* are the most promising organisms [58]. The crude acid hydrolysate was inhibitory to all strains of yeasts, even at dilute hydrolysate concentrations. Strain acclimatization and chemical pretreatment resulted in a marked increase in utilization of substrates in acidic crude hydrolysate. In an attempt to develop a xylose fermenting yeast for industrial ethanol production, UV light-induced mutants of *Pachysolen tannophilus* have been isolated, which grew faster on xylose (Table 3). The central metabolic pathway in yeast is given in Figure 2(a).

Bacteria

The apparent front runners in terms of performance are recombinant enteric bacteria. Researchers at the University of Florida have developed a number of highly productive enteric bacteria by cloning the pyruvate decarboxylate (pdc) and alcohol dehydrogenase (adh) genes from *Zymomonas mobilis* [64-66]. These organisms produce ethanol as their primary fermentation product. Tolan and Finn 1987 transformed *Klebsiella planticola* ATCC 33531 with multicopy plasmids containing the pdc gene inserted from *Z. mobilis* and expression of the gene markedly increased the yield of ethanol to 1.3 mol per mol of xylose, or 25.1 g/L [60] Concurrently, there was significant decrease in the yield of other organic by-products (i.e. formate, acetate, lactate, and butanediol). Ethanologenic strains of both *E. coli* and *Klebsiella oxytoca* have been constructed. The recombinant *E. coli* was used for ethanol production from xylose by Ohta, Beall, Mejia et al 1991 [65] and final ethanol concentration was in excess of 40 g/L with an yield of 0.48 g of ethanol per g xylose, the maximum volumetric productivity per hour being 2.0 g/L which is almost twice that previously obtained with ethanologenic *E. coli*. The hybrid gene, the truncated xylanase gene (xynZ) (from *Clostridium thermocellum*) fused to N terminus of lacZ, was expressed at high levels (25–93 mU xylanase per mg of cell protein) in ethanologenic strains of *E. coli* KO11 and *Klebsiella oxytoca* M5A1 (pLOI555) [66]. Using these recombinant strains, a two-stage process was evaluated for the fermentation of polymeric feedstocks to ethanol: the harvested cells containing xylanase was added to xylan solution at 60°C, hereby releasing xylanase for saccharification, and after cooling, the hydrolysate was fermented to ethanol with the same organism at 30°C. The recombinant M5A1 showed approximately 34% of the maximum theoretical yield of ethanol and this yield appeared to be limited by the digestibility of commercial xylan rather than by a lack of sufficient xylanase or by ethanol toxicity. Ethanol production from xylose with high efficiencies (in some cases nearly 100%) was also reported with recombinant *E. coli* [66]. The maximum final ethanol concentration was 56 g/L and volumetric productivity of up to 1.41 g/L h ethanol was obtained. An ethanologenic xylose fermenting *Z. mobilis* strain also has been developed [67] (Table 3). The central metabolic pathway in bacteria is given in Figure 2 (b).

Table 3: Examples of xylose-utilizing microorganisms

Microorganisms	Comment	References
Pichia stipitis NRRL Y-7124, Y-11 544, Y-11 545	NRRL strain Y-7124 utilized over 95% xylose based on 150 g/L initial concentration. Produced 52 g/L of ethanol with a yield of 0.39 g ethanol per g xylose.	[59]
Klebsiella planticola ATCC 33 531	Carried gene from *Z. mobilis* encoding pyruvate decarboxylase. Conjugated strain tolerated up to 4% ethanol.	[60]
Pichia stipitis NRRL Y-7124 (Flocculating strain)	Maximum cell concentration of 50g/L. Ethanol production rate of 10.7 g/L/h with more than 80% xylose conversion. Ethanol and xylitol yield of 0.4 and 0.03 g/g xylose.	[61]
Escherichia coli (s171)	Yields with *P. tanophilus* strains m and s higher than *E. coli*.	[62]
Zymomonas mobilis	Maximum ethanol conc. of 24.1 g/L with strain of *P. tanophilus* using 200g/L xylose.	[62]
Pachysolen tannophilus *Saccharomyces cerevisiae* ATTCC 24 860	Co-culture of *S. cerevisiae* and strains resulted in the best ethanol yield.	[62]
Saccharomyces cerevisiae CBS 1200 and *Candida shehatae* ATCC 24 860.	Co-cultures of yeast strains utilized both glucose and xylose. Yields of 100 and 27% on glucose and xylose, respectively.	[63]
Pichia stipitis NCIM 3498 and *Saccharomyces cerevisiae*-VS_3	Co-culture of yeast strains used to produce15.0±0.92 g/L ethanol from hemicelluloses hydroysate.	[64]

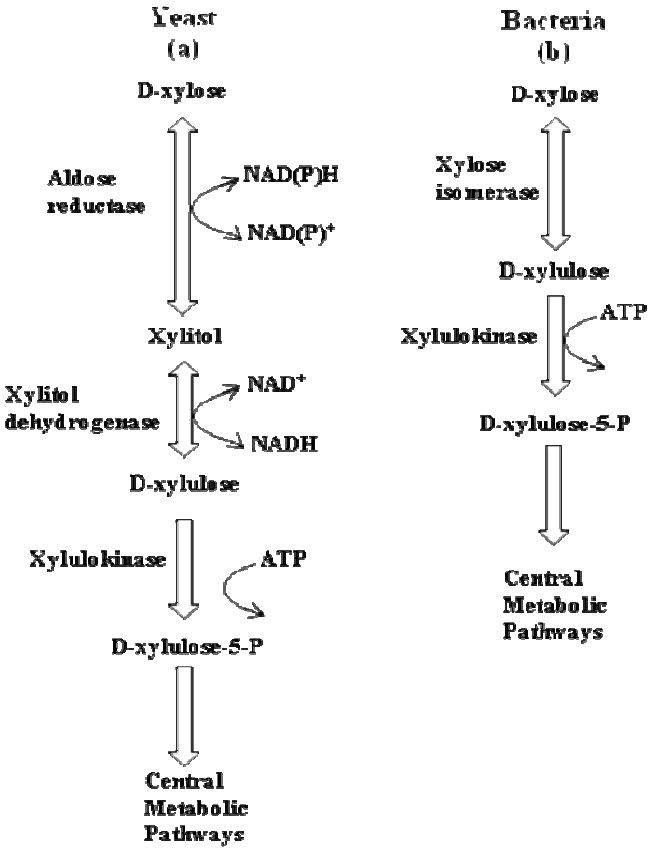

Figure 2: D-Xylose utilization pathways in yeast and bacteria.

Filamentous Fungi

Aerobic filamentous fungi tolerate industrial substrates well and ferment pentose sugars [68], albeit with low rates of sugar consumption and product formation [69]. Also, some species of

anaerobic filamentous fungi produce ethanol, in addition to acids and hydrogen [70, 71]. The poor ethanol tolerance of these organisms is a drawback in industrial applications.

Increasing Production Performance

A variety of factors influence the xylose fermentation performance. For wild-type xylose fermenting yeasts, aeration is one of the dominant factors influencing performances. Beside this secondary factors affecting the performance of yeasts are medium composition, pH and temperature. To achieve high-yield ethanol production, media must be formulated to optimize the levels of vitamins and trace minerals, as well as the type of nitrogen source.

Oxygen Supply

D-xylose catabolism by yeasts leads to simultaneous productions of: (1) Cell biomass, through the tricarboxylic acid cycle, and (2) ethanol, through the fermentative pathway. The relative proportions of cell biomass and ethanol are dependent on the rate of oxygen transferred to the culture. This mechanism is similar to the 'Pasteur effect'. Under anaerobic conditions, yeast growth is severely restricted and xylose is preferentially converted into ethanol; in the meanwhile small amounts of xylitol are produced in relation to a NAD^+ cofactors deficiency.

The first two reactions of the D-xylose catabolic chain in *Pichia stipitis* are the major limiting steps of the fermentation. In *Pichia stipitis* as in other yeasts, xylose reductase is mainly NADPH-linked, whereas xylitol dehydrogenase is predominantly NAD-linked. Since the xylose catabolism does not provide a NAD^+ surplus, the resulting NADH accumulation leads to a redox imbalance under anaerobic conditions, which delays the reaction. This phenomenon, in turn, frequently results in the excretion of xylitol and concomitant low ethanol yields at low production rates. The presence of exogenous hydrogen acceptors, like oxygen, is one of the keys of the xylose catabolism in these yeasts. This regulatory mechanism is referred to as the Kluyver effect [72]. A low transfer rate of oxygen permits circumventing the imbalance of NAD^+/NADH that occurs in anaerobic conditions. In oxygen limited conditions, ethanol production by *Pichia stipitis* is consequently stimulated and xylitol excretion is reduced [61]. In contrast, increasing the oxygen transfer rate tends to favour cell production and is detrimental to ethanol production. At high oxygen transfer rates, according to the Pasteur Effect the carbon flows preferentially through the tricarboxylic acid cycle. In these conditions both the yield and the specific production rate of cells are enhanced, thus reducing the yield and rate of ethanol production. No ethanol is produced under strictly aerobic conditions.

Substrate Concentration

Substrate tolerance of *Pichia stipitis* grown on D-xylose is enhanced in presence of oxygen [73]. The optimum substrate concentration (D-xylose) is 20 g/L, below this level increase in initial substrate concentration tends to increase the ethanol production. However, the opposite effect observed above this level of substrate concentration [74]. This is owed to negative influences exerted on the yeasts both by the substrate and the ethanol produced, this latter effect being predominant [75].

Temperature and pH

High conversion is favoured by lower pH and moderate temperature. Ethanol production in weak acidophiles such as yeast may improve at lower pH because of transmembrane difference in pH as driving force for symport-based xylose transport increases when pH is lowered [76,77]. When proton symport occurs, intracellular xylose concentration is influenced by external pH. Although higher temperature does not have a pronounced effect on conversion performance, particularly

for *P. stipitis*, the inhibitory effect of ethanol increases with increasing temperature. Higher final ethanol concentrations are achieved if the temperature is reduced as ethanol accumulates [76].

Maximum rates of D-xylose fermentation and growth of *Pichia stipitis* occur at 30˚C whereas the optimal pH lies between 4.5-5.5 pH units [76]. Temperature and pH values have separate influence on the fermentation parameters and affect both the yield and rate of xylose conversion. Temperature and pH have been identified as two important factors influencing xylose conversion by recombinant *E. coli* bacteria [57, 78, 79]. Performance of recombinant *E. coli* is best at near-neutral pH and falls off below pH 6. Although initial productivities increase at higher temperature, maximum yield is observed at lower temperature because of reduced ethanol inhibition. Recombinant *E. coli* performs well using inexpensive nutrient sources such as corn steep liquor [79].

Commercialisation

Currently, there are few full-scale or demonstration plants for the production of bioethanol from wood using the enzymatic hydrolysis process. The University of Arkansas pilot plant is based on the SSF process for cellulosic biomass conversion to ethanol built in early 1980s. Iogen (Ottawa, Ontario, Canada) has built a commercial demonstration plant based on this technology for the conversion of agricultural residues to ethanol [80]. The demonstration plant is designed to prove the feasibility of Iogen's EcoEthanol^{TM} process by validating equipment performance and identifying and overcoming production problems prior to the construction of larger plants. Iogen's EcoEthanol process uses an enzyme hydrolysis to convert the biomass into sugars. These sugars are fermented and distilled into ethanol fuel using conventional ethanol distillation technology. In 1997, they partnered with Petro-Canada to produce cellulose–ethanol beginning with a 1-million-gallon-per-year ethanol demonstration facility, located at Iogen's headquarters in Ottawa, using corn stover and switch grass (Iogen Corporation, Marketing and Communications). The French engineering firm Technip and Institut Francais du Petrole constructed a pilot plant in Soustons, France to enzymatically convert cellulose based on Stake process. In 2005, a Swedish plant in Örnsköldsvik started producing ethanol utilising sawdust as raw material. BC International has patented new organisms that have the ability to ferment five-carbon sugars to ethanol as well as offering the opportunity to hydrolyze the cellulose with enzymes [81]. Commercialization is ongoing with a large scale plant under construction.

CONCLUSION AND FUTURE DIRECTION

In order to maximize the ethanol yield from lignocellulosic feedstocks it is essentially required that the hemicellulose fraction must be utilized along with the cellulose in order to obtain an economically viable conversion technology. The efficient pretreatment/hydrolysis process for the recovery of maximum amount of fermentable sugars (hexose and pentose) with the minimum or no toxic chemicals is the major challenge and requires advance biotechnological approaches to conquer this problem. Another bottleneck is the complete bioconversion of both type of sugars released from the lignocellulosic biomass to ethanol and recombinant DNA technology is the only tool to develop such microbial strain that can utilize both hexose and pentose sugars to produce ethanol. Further, integrating production process - the design of fermentation and downstream separations as a single, integrated process can make the overall process economically practicable.

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