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Assessment of pretreatments and enzymatic hydrolysis of wheat straw as a sugar source for bioprocess industry

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Abstract
Environmental concerns and rising oil prices have led to development of biofuels from crop residue lignocelluloses, among which wheat straw is an important feedstock used in leading commercial bioethanol processes. Lignocellulose is structured in a way that makes direct bioconversion of biomass into sugars by hydrolytic enzymes difficult and unfeasible, requiring a pretreatment step. Common biomass pretreatment technologies are assessed for potential application in obtaining fermentable sugars of wheat straw. Current outlook, challenges and opportunities on enzymatic hydrolysis of lignocellulose are also presented.

Keywords: Pretreatment, wheat straw, Enzymatic hydrolysis, Saccharification.

1. Introduction

Industrial bioconversion of renewable resources is a promising alternative to petroleum-based chemical synthesis [1]. In this context, lignocellulosic biomass is an important renewable source of energy that has the potential to supply 20%-100% of the world's total annual energy consumption [2]. Lignocellulose-based biorefineries are viewed as the trend of the future that would convert biomass into products falling into traditional petrochemical and future biobased markers [3]. Out of these products biofuels are of the utter most importance. In the United States, transportation biofuel production is currently dominated by first generation biofuels: maize grain ethanol and soybean biodiesel which are used as fuel additives and are short in supply [4]. Environmental and economic concerns associated with the use of fossil fuels have led to surge in development of second generation biofuels derived from lignocellulosic feedstock to transform transportation sector into a green infrastructure[4, 5]. Many feedstock are available for conversion such as crop residues (e.g. corn stover, wheat straw), dedicated energy crops (e.g. switch grass, poplar trees), forest residues (e.g. sawdust) and municipal solid waste (e.g. waste paper) [6, 7]. Among these, crop residues such as wheat straw and corn stover, and switch grass are thought to be of primary importance due to high availability and efficiency of conversion [8]. Lignocellulose feedstock biorefinery would consist of the four main stages: pretreatment, enzymatic hydrolysis, fermentation, and distillation. Besides feedstock, the costs of which can be minimized by focusing on agriculture residue, pretreatment to increase the susceptibility of biomass to enzymatic attack and enzymatic hydrolysis to release constituent sugars from biomass are the most expensive steps and require special attention [9]. Wheat straw has gained considerable utilization in commercial pilot plant bioethanol production [8, 10]. The purpose of this review is to examine the most common biomass pretreatment technologies with

respect to wheat straw as a feedstock of application. Enzymatic hydrolysis step is also given consideration.

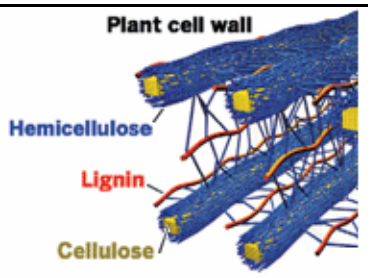
Global annual production of wheat is 529 Mton with a global production yield of 3.4 dry Mg/ha. Asia (43%), Europe (32%), and North America (15%) are the leading regions of production. With the residue to crop ration of 1.3, around 687 Mton of wheat straw is produced annually in the world. Proper soil management is required for this biomass feedstock to be sustainable, and so some of the crop residue must be left on the field as ground cover to regenerate soil and reduce erosion. Assuming ground cover of 30% determined by US Department of Agriculture as a good tillage conservation practice, this makes 481 Tg of wheat straw annually available for conversion into related biofuels and bioenergy products. The bioethanol potential of this residue is 141 GL and if lignin is burned in power plants after bioprocessing than it would produce 141 TWh of electricity [11]. Currently commercial ethanol from wheat straw is produced by Iogen in Canada and DONG Energy in Denmark [8,10]. Although the focus of utilization of this residue would lie on fermentative applications for production of bioethanol or biobutanol to affect economic and environmental solutions to rising oil prices and automotive emissions, wheat straw has a whole spectrum of other useful applications. It can be used for animal feed [12, 13], production of pulp and paper [14], strawboards [15], textiles and composites [16], plastics [17] and removal of metals in wastewater industry [18, 19].

2. Composition of wheat straw

On average, wheat straw consists of 33-40% cellulose, 20-25% hemicellulose, 15-20% lignin [20], 2-7% ash, 5% extractives, few pectic and mannan compounds and structural proteins [21]. The chemical composition fluctuates among different wheat straw varieties (Table 1). At the same time, there are significant differences in the composition between the botanical components (i.e. steam, leaf, and node) of straw. The stems account for 50-60% w/w and are richer in cellulose while containing less ash. The leaves that account for around one third of biomass fraction contain more ash and nodes are higher in lignin [21, 22]. Wheat straw contains higher levels of cellulose and hemicellulose and lower amount of lignin than corn stover [23], making this type of biomass a more efficient feedstock for fermentable applications as a richer sugar platform of dry feed would result higher biofuel yields in downstream fermentation processes (Table 2).

Table 1. Chemical composition of wheat straw from different studies along with a representation of a general structure of lignocellulosic biomass

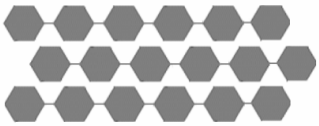
Country	Ref	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
USA	[66]	48.6	27.7	8.2	6.7
Canada	[88]	34.5	21.3	17.5	2.7
Denmark	[63]	35.0	22.3	15.6	6.5
Spain	[65]	37.6	24.7	17.4	4.8
Netherlands	[36]	36.3	21.1	25.5	6.7
Korea	[73]	37.6	24.7	19.6	2.5



2.1 Cellulose

Cellulose is a homopolymer of glucose linked by β-1,4-glucosydic linkages with a degree of polymerization of 500~15000. In plants, cellulose chains are bundled together by hydrogen bonding into semicrystalline microfibrils containing the crystalline allomorphs, cellulose I alpha and I beta [24]. Factors that influence cellulose hydrolysis by cellulase enzymes include degree of polymerization, crystallinity, accessible surface area, and the presence of lignin [25] and structural polysaccharides [26, 27]. Wheat straw contains cellulose I beta allomorph with 40% crystallinity [28]. Low crystallinity of wheat straw cellulose makes it a good substrate for enzymatic saccharification [25] as well as a suitable host polymer for preparation of cellulose derivatives [28]. In epidermis cell walls, cellulose microfibrils linked together by amorphous serrated regions arrange longitudinally, while random arrangement is observed in the parenchyma cell walls [29]. Ultrastructurally, cellulose microfibrils are embedded into hemicellulose matrix where it is supported by hydrogen and covalent bonding to hemicellulose polysaccharides that are wrapped by lignin [30].

Table 2. Overview of structural characteristics of wheat straw lignocellulose

Component	Cellulose	Hemicellulose	Lignin
Weight fraction	33-40%	20-25%	15-20%
Schematic illustration			
Structural features	-cellulose chains of glucose monomer of DP 500-1500 bonded into semicrystalline microfibrils ~40% crystallinity -supported by hemicellulose via hydrogen and covalent bonding	-70-90% xylan, rest arabinan -amorphous, branched polymer with DP of 70-200 -xylan backbone substituted by arabinan, uronic acids and acetyl groups -bonded to lignin through ferulic or p-coumaric acid bridges	-p-hydroxyphenyl-guaiacyl-syringyl (H(5%)-G(49%)-S(46%)) phenolic monomers -highly amorphous and branched forming a protective shell around the sugar platform
Factors affecting enzymatic hydrolysis of cellulose	-degree of crystallinity, DP, accessible surface area -structural hindrance by hemicellulose and lignin components	-xylan substituents as well as strong interaction with lignin limit enzymatic conversion of xylan	-structural barrier -unspecific binding of enzymes

2.2 Hemicellulose

Hemicellulose is a heteropolymer of pentose (D-xylose, L-arabinose) and hexose (D-mannose, D-glucose, D-galactose) sugars and sugar acids that vary in composition depending on the plant species [31]. The degree of polymerization of the majority of hemicelluloses is 70-200 monosaccharide units [32]. Hemicellulose fills the gap between lignin and cellulose and its solubilisation is directly linked to an increase the biomass porosity [33, 34]. Hemicellulose's highly branched and amorphous structure makes it the easiest component to solubilise during thermo-chemical pretreatments, solubilisation of hemicellulose begins at 150ºC under neutral conditions and as low as 120ºC in dilute presence of acid catalyst [35]. Wheat straw hemicellulose is primarily arabinoxylan containing 70-90% xylan, the rest being arabinose with minor amounts (<0.6%) of mannose, galactose, and glucose [36, 37]. A polymer of xylose, xylan backbone is substituted by arabinan, uronic acids and acetyl groups [38]. Hemicellulose is associated with lignin through lignin-carbohydrate complex that consists of either etherified or esterified ferulic or p-coumaric acid bridges bonded mostly to arabinan, and about 1% wheat straw lignin is directly linked to uronic acid side chains by ester bonds [38, 39]. The content of esterified and etherified *p*-coumaric acids is 3.78 and 1.72% respectively, and the content of esterified and etherified ferulic acid is 1.02 and 2.2%, respectively [23]. Dimerization of esterified phenolic compounds may also lead to cross linking of xylan [38].

2.3 Lignin

Lignin is a tridimensional polymer of phenylpropane alcohols (*p*-hydroxycinnamyl [H], guaiacyl [G], syringyl [S]) linked through both ether and carbon-carbon bonds [40]. Wheat straw lignin is also called *p*-hydroxyphenyl-guaiacyl-syringyl (H-G-S) lignin and contains the three components in 5, 49, and 46% of respective proportions [23]. Different species of wheat straw lignin oligomers are formed from different types of monomer coniferyl residues. The main repeating units are formed from two adjoining di-coniferyl residues that contain an intermediate five-membered furan-like ring, formed as a result of covalent and ether bonding between the two di-coniferyl units [40]. Guaiacyl unit is the connector between lignin and hemicellulose and the main component of condensed lignin. Extracted lignin from fractionation of wheat straw can be utilized for production of valuable food and industrial products such as vanillin, ferulic acid, and optically active monolignol dimmers [23]. Wheat straw contains significant amounts of extractable ferulic and p-coumaric acids (~3.6 mg/g) compared to flax sheaves (0.2 mg/g) and separation of these acids should be included in an integrated wheat straw biorefinery to improve the

cost effectiveness of the process [23]. Ferulic acid can be used for vanillin production, UV protection in cosmetics, and as a food antioxidant [41]. *p*-Coumaric acid displays antioxidant and anti-inflammatory properties and it was recently found to be a potential dietary supplement for primary prevention of vascular disease [42, 43].

2.4 Pectin

Pectin is a complex structural hetero-polysaccharide composed of galactouronic acid residues substituted by methoxyl esters and sugars. Pectin content influences biomass porosity and buffering capacity. Wheat straw contains 5% of low-methoxy partially acetylated pectin with 25% galactouronic acid content. Pectin extracted from citrus or apple peel is used in food industry as a gelling, stabilizing, or thickening agent. However, due to presence of acetyl groups, low molecular weight, and low viscosity wheat straw pectin does not possess these gelling properties and it find applications in low-caloric, high-fiber beverages. Pectin is also rapidly finding uses in medical field where studies have shown it be beneficial for regulating gastrointestinal infections, blood cholesterol, and glucose adsorption [44, 45]. Therefore, extraction of pectin from wheat straw could also be considered in an integrated wheat straw biorefinery to buffer overall process economics.

2.5 Wax

Wax consists of primarily long chain fatty acids and fatty alcohols, sterols and alkanes. Natural waxes have a wide range of industrial uses in cosmetics, polishes and coatings, pharmaceuticals, and insecticides. Wheat straw contains around 1% wax by weight that covers the outside surface of the plant material making it potentially feasible to remove this component independently in a preceding extraction stage [15, 46].

2.6 Ash

Ash represents the mineral content of biomass that depends on the soil and environmental conditions. In general, the ash content of crop materials is significantly higher than that of wood [34]. Silica accounts for around 80% of wheat straw ash, the rest being metals such as sodium and potassium [47]. Burning biomass for production of electricity rather than using it as a feedstock for production of biofuels would lead to its greatest utilization as an energy source [48]. However, the high content of alkali metals in straw, which lead to corrosion deposits in boilers, makes this type of biomass not well suited for combustion [49]. Besides combustion, ash can lead to fouling of process equipment during pretreatment [22]. Degree of lignification and ash content are direct indicators of quality of biomass feedstock. As wheat straw leaves contain more ash than other components and nodes are high in lignin, while stems are richer in cellulose, physical separation of botanical components of wheat straw components can improve feedstock quality and reduce the amount of straw used for ground cover [22].

3. Pretreatment of Lignocellulose

The highly recalcitrant structure of lignocellulose creates mass-transport limitations for penetration by chemical or biological catalyst [50]. A pretreatment is required to break up the recalcitrant structure of lignocellulose and improve the accessibility of hydrolytic enzymes to their substrates (Figure 1). For instance, cellulose conversion to sugars of untreated wheat straw could reach up to a maximum of 30% which is not sufficient to produce enough fermentation products to recuperate costs [51]. In studies, mechanical particle size reduction to increase accessible surface area of biomass is usually followed by physiochemical pretreatments.

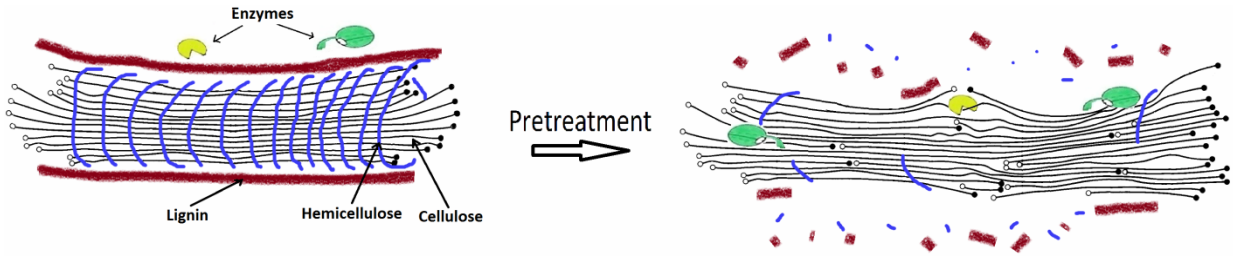


Figure 1. The main goal of pretreatment is to increase the accessibility of cellulose to cellulolytic enzymes in subsequent enzymatic hydrolysis stage

4. Mechanical pretreatment

Milling, grinding, or cutting reduces biomass particle size, and increases bulk density and accessible surface area, which improves the efficiency of subsequent processing by decreasing transportation costs, improving flow properties, and minimizing heat and mass transfer limitations [52, 53]. Particle size reduction also causes reduction in crystallinity and mean degree of polymerization [54]. Biomass characteristics, screen size, moisture content affect the specific energy consumption during milling [55]. Grinding performance of knife and hammer mill of wheat straw are reported by [53,56]. Knife milling was studied for larger screen size (12.7-50.8 mm), and optimum total specific energy consumption for wheat straw was 37.9 MJ/Mg for screen size of 25.4 mm. Hammer milling was studied for smaller screen size (3.2mm) and it was preceded by knife milling to screen size of 25.4mm. Optimum total specific energy during hammer milling of wheat straw was 125.1 MJ/Mg for 3.2 mm screen (Table 3). The two operations can thus be performed in a sequence. Wheat straw displayed higher specific energy consumption than corn stover and switchgrass due to flexible, slippery and less brittle nature of straw. However wheat straw particles after hammer milling were coarser and deemed more suitable for bioprocessing [53]. Finer particles can be obtained by further ball-milling the straw for long period of time (e.g. 14 hours) to obtain direct enzymatic hydrolysis yields of up to 80%. However, the conversion of substrate by the enzymes was about twice as long compared to the substrate pretreated by chemical means [57] and this operation may go beyond the threshold of economic feasibility due to increased energy consumption

Lignocellulosic biomass requires much more energy to achieve the same particle size reduction than coal [53]. The high energy consumption can make finer mechanical pretreatment detrimental to the overall process economics and the general opinion is that this costly unit operation should be avoided on an industrial scale [35, 58]. However, an economic analysis is required before such conclusion is made as efficiency improvements of downstream processes may outweigh the costs of milling which has been demonstrated in commercial pilot plant operations [8].

Table 3. Specific energy consumption during milling of wheat straw

Mechanical Pretreatment	Screen Size	Specific Energy Consumption
Knife Milling	25.4 mm	37.9 MJ/Mg
Hammer Milling	3.2 mm	125.1 MJ/Mg

5. Acidic pretreatments

Acid pretreatments involve the use of high temperature steam or water with or without acid catalyst. During the pretreatments, internally produced acids also serve as a catalyst ("autohydrolysis"). Acid catalyzed hydrolysis and partial degradation of hemicellulose which increases biomass porosity and change in lignin structure are the major events that occur during these pretreatments. Sugar degradation products such as furfural and HMF, and phenolic compounds may be toxic to some fermentative bacteria [58]. Pretreatments that fall into this category are steam (explosion), liquid hot water and dilute acid pretreatment.

5.1 Steam explosion

Steam explosion is the most effective pretreatment of lignocellulosic biomass that is currently used for commercial ethanol production from wheat straw [8, 10]. The process involves holding biomass at high temperature and pressure followed by a rapid decompression (explosion). The explosion causes defibration, separation of individual fibers and cell types, while high temperature acidic environment cause solubilisation and hydrolysis of hemicellulose into monomers, solubilisation and redeposition of lignin globules onto the fiber surface [59, 60]. The pretreatment was found to increase the lignin and cellulose content of the solid fraction by 100% and 22.9% respectively while the content of hemicellulose was reduced by 59.2% compared to untreated straw [61]. Ballesteros et al. studied steam explosion pretreatment with acid- (0.9% w/w H_2SO_4) and water-impregnated wheat straw. Maximum cellulose and hemicellulose sugar recovery after enzymatic hydrolysis occurred for acid-impregnated straw pretreated at 180°C for 10 min. Maximum glucose yield of 85% after enzymatic hydrolysis was obtained when wheat straw was pretreated at 180°C for 10 min or 190°C for 5 min, however at temperatures of 190°C and higher excessive xylose degradation to furfural took place [62].

5.2 Liquid hot water (hydrothermal)

Liquid hot water pretreatment has the advantage of using no added chemicals and minimizing hemicellulose degradation. During the process, individual fibers and cell types are separated, hemicellulose is removed, and lignin is redeposited on the fiber surface in the form of globules but to a smaller degree than in steam explosion [59]. Around 80-90% of solubilised xylan and around half of solubilised arabinan are present in oligomeric form. Xylan oligomers of more than 15 units can, however, adsorb back onto cellulose and obstruct cellulose hydrolysis [63]. pH can be controlled to further minimize the formation of sugar monomers [64]. Xylose and glucose yields occur at two different optima suggesting the feasibility of a two stage pretreatment [65. In a flow through pilot scale reactor, pretreatment with hot water at 195-200 °C gave 54-56% xylan yield, and 68-72% glucose yield after enzymatic hydrolysis [49]. The major challenge facing this pretreatment method is to minimize the usage of water to make to process economically feasible for industrial scale operation.

5.3 Dilute acid

Dilute acid pretreatment is essentially hot water pretreatment with the addition of an acid catalyst, usually less than 1% v/v H_2SO_4 [66]. During dilute acid pretreatment hemicellulose is removed and hydrolyzed into monomers and solubilised lignin precipitates onto biomass [35]. To maximize the recovery of xylose, lower temperatures (120ºC) and longer times (1hr) are required, while cellulose digestibility suffers by up to 50%. At higher temperatures (up to 180ºC) and shorter pretreatment time (15min), cellulose yield is at a peak while xylose yield is reduced by one third due to sugar degradation reactions catalyzed by sulfuric acid. The corresponding total sugar yields at the two optima were 75% [66]. The wide difference in optima for the recovery of the two sugars makes it difficult to obtain high overall sugar yield in one stage, and a maximum of 84% total sugar was obtained yield after enzymatic hydrolysis of microwave-assisted dilute sulfuric acid pretreatment (160ºC, 10 min, 0.5% H_2SO_4 w/v) of wheat straw [67]. Various organic acids, such as fumaric and maleic acids can be used instead of sulfuric acid to reduce sugar degradation reactions catalyzed sulfuric acid while attaining nearly identical sugar yields [36]. Optimization of dilute maleic acid pretreatment of wheat straw showed a nearly stoichiometric glucose yield after enzymatic hydrolysis and 90% xylose yield at 170 °C, 50 min, 89 mM maleic acid. When costs were taken into account the optimum pretreatment conditions were 170 °C, 50min, 46 mM maleic acid resulting in 85% glucose and 80% xylose yield [36].

6. Alkali pretreatments

Alkali pretreatments use either mineral (i.e. lime, NaOH) or organic (i.e. ammonia) catalyst to solubilise both lignin and hemicellulose. Compared to acidic pretreatments, operating conditions are mild and sugar degradation is minimal, and no inhibitory compounds are formed. Common alkali pretreatments studied are lime, ammonia percolation and ammonia fiber expansion (AFEX).

6.1 Lime

Lime pretreatment is a promising pretreatment method of lignocellulose bioprocessing due to low cost of lime ($0.06/kg), safety, and easy recoverability. The pretreatment has been shown to be effective at enhancing enzymatic digestibility of wheat straw while producing negligible inhibitors [68]. During lime pretreatment lignin is solubilised and a complete deacetylation of xylan occurs which leads to swelling of the biomass. Minor "peeling" of cellulose and hemicellulose also occurs [69]. Optimum sugar yields occur at high temperatures (85-135 °C) and short pretreatment times (1-3h) or low temperatures (50-65 °C) and long pretreatment times (24h) [70]. Optimum lime loading is 0.1g $Ca(OH)_2$/g dry biomass[68, 70]. In a recent study by Saha and Cotta, lime pretreatment of wheat straw gave 82% total sugar yield [68]. An integrated pilot-scale study of lime pretreatment of wheat straw for bioethanol production including conversion of side streams to solid fuel and biogas was done by Maas et al., demonstrating the feasibility of such bioprocessing plant configuration [71]. Oxidative lime pretreatment results in substantially higher delignification (up to 90%) as compared to non-oxidative conditions (around 50%) although sacrificing glucan and xylan recovery [72]. Lime pretreatment of wheat straw at oxidative conditions is not reported, however it may prove effective for straws with higher lignin content.

6.2 Ammonia percolation

Ammonia percolation pretreatment of wheat straw effectively removes 60-70% lignin and around 50% hemicellulose and leaves a highly digestible (up to 95%) solid fraction essentially free of fermentation

inhibitors. High temperature (140-170 °C) is required for effectiveness while pretreatment time can be relatively short (10-30 min) as it was less significant for delignification [73]. The process is made continuous by recycling ammonia.

6.3 AFEX

Ammonia Fiber Expansion (AFEX) is unique method for pretreatment of biomass resulting in unique effects [74]. During AFEX, biomass is contacted with aqueous ammonia at moderate temperatures (80-150 C) and pressure (200-400 psi) for 5-30 min followed by explosive decompression [13]. The process results in cellulose decrystallization and opening of fibrous structure, hemicellulose prehydrolysis and migration to the exterior of cell walls [75]. As opposed to other alkali pretreatments, lignin is fragmented and remains in the substrate rather than being degraded and removed [76]. Enzymatic saccharification of AFEX pretreated wheat straw is not reported in the literature, however, it showed recent improvements for corn stover resulting in minimum ethanol selling price of $1.03 per gallon [77]. The new AFEX process uses an innovative quench system to recover ammonia as compared to traditional distillation and recompression, and minimum amount of ammonia (0.3 kg/kg biomass) and water (0.25kg/kg biomass) to improve process economics. Bals et al. found that AFEX improved ruminant digestibility of wheat straw by 63% implying that pretreatment seems to give positive results for the feedstock [13].

7. Oxidative pretreatments

Oxidative pretreatments such as alkaline peroxide, wet oxidation and ozonolysis use radicals to selectively degrade lignin's phenolic structure. While lignin is lost, selective lignin degradation allows these pretreatments to produce substrates rich in cellulose and hemicellulose which can advantageous in terms of product yields, as compared to substrates that contain lignin, for subsequent solid state simultaneous saccharification and fermentation processes that have limited solids handling capacity.

7.1 Alkaline peroxide

Alkali peroxide pretreatment is an effective method of pretreatment for wheat straw at low temperature that results in nearly stoichiometric enzymatic hydrolysis yields and minimal degradation of sugars and formation of toxic compounds [78-80]. H_2O_2-derived radicals degrade lignin into low molecular weight carboxylic acids which accounts for lack of toxicity of the resultant liquor, and seems to be a feasible way of utilizing lignin as a source of these chemicals. The optimum pH for delignification is 11.5 and the loading of hydrogen peroxide 0.25 w/w. The supernatant fraction from the pretreatment can be recycled at least six times. Increasing the temperature above ambient did not have any effect on the final conversion of cellulose by enzymes after 24 hours. At 25 C pretreatment of wheat straw resulted in one half of lignin and most of the hemicelluloses to be solubilised. Operating at ambient temperature has the advantage of eliminating heating requirements for the process while the effect of long pretreatment times on capital costs has yet to be evaluated [80].

7.2 *Wet oxidation*

Wet oxidation is an effective fractionation method for wheat straw during which the crystalline structure of cellulose is opened, hemicellulose is solubilised, and lignin is decomposed to CO_2, H_2O, and carboxylic acids[81]. The pretreatment also results in slight formation of phenols and 2-ferulic acid that may inhibit downstream fermentation processes [82]. Georgieva et al. studied wet-oxidation pretreatment (180-185 C, 15min) of wheat straw at high dry matter (14%) and low enzyme (10 FPU/g cellulose) loading using three oxidizing agents H_2O_2(35% v/v), O_2(12-18 bars), and air(12-18bars). Air was a poor catalyst, while H_2O_2 and O_2 gave similar glucose yields (69%) after enzymatic hydrolysis. However, H_2O_2 solubilised and degraded more xylan (55% yield) than O_2 (77% yield), making air the best oxidative agent for wet explosion wheat straw [83]. Wet oxidation of wheat straw by using H_2O_2 in a flow through pilot plant scale reactor showed good results on par with Na_2CO_3 and liquid hot water [49].

7.3 Ozonolysis

Pretreatment by ozone solubilises and degrades lignin and slightly solubilises hemicellulose with the advantage of not producing any known inhibitors for subsequent enzymatic and fermentation processes. Ozonolysis of wheat straw was shown to be effective at removing up to 35% of lignin and improving its enzymatic digestability by up to 50% compared to untreated material, with a maximum enzymatic

hydrolysis yield of glucose at 88.6%. The pretreatment was more effective for wheat straw than rye straw; possibly related to higher lignin content of the latter [51].

8. Fractionation pretreatments

Fractionation pretreatments break up biomass into its constituent components hemicellulose, cellulose and lignin which are then recovered by separation/extraction. Organosolv pretreatment removes hemicellulose and lignin from cellulose microfibrils while Ionic liquids and phosphoric acid pretreatment cause additional dissolution of cellulose. As opposed to other pretreatments, neither lignin nor hemicellulose are degraded nor is lignin structure altered which allows for extraction of high quality lignin.

8.1 Organosolv

Organosolv pretreatment occurs in the temperature range of 100–250 °C using a number of organic solvents (i.e. methanol, ethanol, glycerol, ethers, phenols etc). Various acid and alkali can be added as catalyst. The pretreatment results in substantial hemicellulose and lignin solubilisation, while cellulose remains intact, making possible the full separation of the components upon fractionation [84]. A nearly full conversion of cellulose to glucose can be achieved by enzymatic hydrolysis thereafter. Pretreating wheat straw with aqueous glycerol with the liquid to solid ration of 20g/g at 220 C for 3hrs resulted in removal of 70% of hemicelluloses and 65% lignin and a subsequent 90% enzymatic hydrolysis yield [85]. Aqueous methanol percolation (1:1 v/v, 1min, 10 ml/min) of wheat straw and a subsequent enzymatic hydrolysis resulted in an overall 90% glucan-to-glucose conversion [86]. The concept of organosolv biorefinery is presented in [84].

8.2 Ionic liquids

Pretreatment with ionic liquids is an effective and environmentally friendly way of dissolving lignocellulose that results in amorphous and porous substrate prone to enzymatic degradation [87]. Ionic liquids are nonflammable and recyclable, and act by hydrogen-bonding with cellulose at elevated temperatures which causes dissolution. Antisolvent such as water can be used to regenerate fibers hemicellulose and cellulose rich fibers, while lignin can be extracted making this pretreatment a good fractionation method[87, 88]. Li et al. obtained a poor enzymatic hydrolysis yield of 54.8% after pretreating wheat straw with [EMIM] DEP at 130C for 30 min. However the hydrolysis time (12h) was shorter than is usually required and cellulase was not supplemented by β-glucosidase [87]. This pretreatment has shown to be very effective at disrupting lignocellulosic matrix of switchgrass resulting in a maximum theoretical yield of glucose after 30h of enzymatic hydrolysis[89].

8.3 *Phosphoric acid*

The use of concentrated phosphoric acid (>83%) has a similar dissolution effect to ionic liquids resulting in an amorphous cellulosic substrate devoid of hemicellulose and delignified to a greater extent [90, 91]. During the pretreatment lignin-carbohydrate bonds and hydrogen bonding between sugar chains are disrupted, cellulose and hemicellulose are weakly hydrolyzed to short fragments and acetyl groups are removed forming acetic acid. Organic solvent such as acetone can be used to precipitate and separate the fractionated biomass. The pretreatment has an advantage of operating at low temperature (50 °C) which capital and operating costs and minimizes degradation reactions. The residual phosphoric acid in regenerated substrate has no inhibitory effects on the sequential hydrolysis and fermentation [91]. Although there is no literature for wheat straw, this pretreatment method showed stoichiometric enzymatic hydrolysis yield for triticale straw after pretreatment with 86.2% phosphoric for 110.5 min at 50 °C. During the pretreatment hemicellulose showed full tendency to solubilise and cellulose and lignin solubilisation reached 25% [90].

9. Biological

White-rot fungi have gained attention for biodegradation of lignocellulose for their ability to secrete phenol oxidases that degrade lignin. Some carbohydrates, especially hemicellulose, are degraded and co-metabolized to provide fuel for and improve accessibility of lignin degrading enzymes. The white-rot fungi can establish synergistic relationship with cellulolytic organisms for complete biodegradation of lignocellulosic wastes [7, 30]. Hatakka obtained 35% enzymatic hydrolysis yield after incubating wheat straw with *Pleurotus ostreatus* for 5 weeks. Oxygen accessibility plays a key role in delignification by

white-rot fungi as whole straw showed better results than milled straw [92]. Combined with lengthy pretreatment times, on an industrial scale this would require large allocation of space and capital equipment, entailing large capital costs. Issues with scale-up, heat build-up, process control, loss of carbohydrates to power delignification and, after all, loss of lignin, indicate that this pretreatment is not feasible for industrial processing of biomass [30]. Fungi would rather be used for local low cost bioremediation projects to treat landfills or lignocellulosic waste that is rare or is unfeasible to transport to biorefineries [7].

10. Supercritical CO_2

Supercritical CO_2 (SCO_2) has been mostly studied as an extraction solvent [93]. Extraction of wheat straw waxes by this technology was done by [46]. Optimum yield occurred at maximum pressure 30 MPa and minimum temperature 40 °C which corresponded to maximum solvent strength. The composition of the extract could also be tailored by adjusting the SCO_2 and 99% of the total extractable wax could be obtained in less than 70 min of extraction time. SCO_2 was also found to be more selective than conventional solvents used in soxhlet extraction [46]. Advantages of using SCO_2 include low cost, environmental compatibility and easy recoverability [93]. This extraction technique was also suggested as a first step in an integrated wheat straw biorefinery. Feasibility of SCO_2 extraction is however hampered by high capital, operating and maintenance costs and research is underway to improve the overall extraction process economics [15].

Besides extractive purposes, SCO_2 can also be used to enhance enzymatic digestability of lignocellulose, but the operating conditions differ between the two pretreatments [46,93]. SCO_2 pretreatment of woody lignocellulose showed optimum at 21 MPa and 165 C with 30 min pretreatment time with subsequent enzymatic hydrolysis yields of up to 85% [93. Similar to other explosive pretreatments, rapid release of carbon dioxide pressure was found to disrupt the structure of cellulose and increase its susceptibility to enzymes by as much as 50% [94]. No studies were done on wheat straw with this pretreatment.

The summary of common pretreatments of lignocellulose shown in Table 4.

Table 4. Summary of common pretreatments of lignocellulose

Pretreatment				
Mechanical	Thermochemical			
Milling: *-Knife* *-Hammer* *-Ball*	Acidic: *-Steam Explosion* *-Liquid Hot Water* *-Dilute Acid*	Alkaline: *-Lime* *-Ammonia Percolation* *-AFEX*	Oxidative: *-Alkaline H_2O_2* *-Wet Oxidation* *-Ozonolysis*	Fractionation: *-Organosolv* *-Ionic Liquid* *-Phosphoric Acid*
Effect on biomass				
-particle size reduction/densification *Ball milling*: -reduction degree of crystallinity, DP	-removal and hydrolysis of hemicellulose -transformation of lignin structure	*Lime & Ammonia Percolation:* -delignification and solubilisation of hemicellulose *AFEX*: -fragmentation of lignin	-selective degradation of lignin to a high degree	-hemicellulose and lignin solubilisation *IL & Phosforic Acid:* -cellulose dissolution
Advantages				
-improve efficiency of downstream processes	-effective and economically viable without use of external acid catalyst	-milder operating conditions -fully recyclable catalyst -minimal loss of sugars	-sugar rich substrate offers potential improvements in fermentation yields	-possibility of integrated biomass biorefinery -dissolved cellulose is highly susceptible to enzymes

11. Future perspective on pretreatment

First and foremost, an effective pretreatment would have to make the entire process economically feasible in the long run. Looking at the enormous tonnages of feed that would be handled in the future,

the use of chemicals would have to be minimized, or they would have to be recyclable to a reasonable degree. Maximize the efficiency of downstream processes, pretreatment has to be effective at disrupting recalcitrant ultrastructure of lignocellulose, minimize loss of sugars and be capable of operating at elevated solids loadings. Although milling of biomass has found use in some commercial pilot plants, due to its high specific energy consumption, it may be outcompeted by processes that bypass this step.
Great progress has been made towards commercialization of steam explosion pretreatment of wheat straw for bioethanol production by DONG Energy, Denmark (Figure 2). At their IBUS pilot plant facility all steps in the process are operated at high dry matter content. Wheat straw is crudely cut into 5-10cm pieces and is impregnated with recycled acetic acid formed during pretreatment before it enters steam pretreatment vessel at 40% dry matter that uses no added chemicals. The pretreated fibers are then loaded into liquefaction reactor at 25-30% dry matter content to improve fluid properties of the substrate and pretreatment liquor rich in hemicellulose derived sugars is sold as cattle feed molasses. Liquefaction is shortly followed by simultaneous saccharification and fermentation (SSF). After SSF, the fermentation broth is distilled to recover ethanol and lignin rich fiber stillage is utilized in boilers to generate steam and power for the process. The amount of solid biofuel generated by the process is more than is required to power the process and so additional profits could be made by selling the excess power generated to an electric grid. The process was bottlenecked by design of particle pumps, to move biomass throughout the process, and gravimetric mixing reactor for enzymatic liquefaction and SSF at high dry matter content.

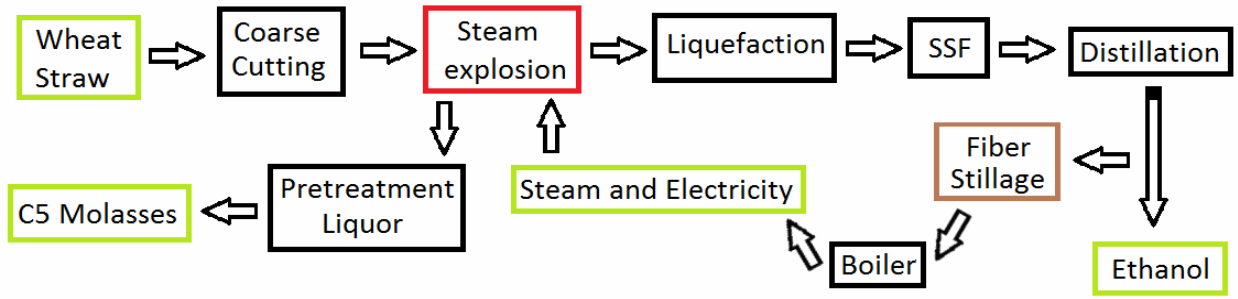


Figure 2. Process flow diagram for IBUS bioethanol production process from wheat straw

Utilizing lignin as a boiler fuel to generate steam and selling excess power generated to utilities is a commercially viable approach, developing lignin as renewable source of biochemicals could bring greater surpluses to the profits of a biorefinery. The use of oxidative pretreatments that rely on degradation of this component would not be feasible as great amounts of this valuable commodity would be wasted. Besides lignin, miscellaneous components of wheat straw such as wax, pectin, and phenolic acids are also of great value and their isolation would be included in an ideal biorefinery. A thorough economic evaluation accounting for these value-added products could turn the economics towards a pretreatment that allows for their extraction away from the steam pretreatments currently used for commercial production of ethanol. In this context, fractionation pretreatments that allow for fractionation of biomass into its constituent components may find greater commercial success in the future when the prices of petrochemicals start rising. An organosolv fractionation process is under development by Lignin Inovation Corporation, Canada, where isolated cellulose is saccharified for fermentative purposes while dissolved lignin, hemicellulose, and extractives are separated for further processing [95]. Additional research is required to evaluate cellulose dissolution pretreatments such as ionic liquids and phosphoric acid pretreatment as they offer greater potential at biomass fractionation and superior kinetic advantages for enzymatic hydrolysis step.

12. Enzymatic hydrolysis: enzyme systems and process overview

Typical cellulose microfibril contains crystalline and amorphous regions and reducing sugars (ends) on one end and non-reducing sugars on the other end with a slight mix of the two sugar chain ends in between. [96] Enzymatic hydrolysis of cellulose microfibrils to release glucose involves synergistic action of three enzymes: endo-glucanase, exo-glucanase and β-glucosidase. Endo-and exo-glucanases are commonly referred to as “cellulases”. Fungal strains of *Trichoderma reesei* are used to produce most commercial cellulase mixtures that also contain some β-glucosidase activity. Cellulases consist of a catalytic domain and a cellulose binding domain (CBD) that regulates docking of cellulases onto the

substrate [97]. Endo-glucanases attack amorphous regions of cellulose releasing short cello-oligomers and creating new chain ends for exo-glucanases. Rapid decrease in DP of cellulose is observed and cello-oligomers begin to dissolve from the substrate after some time. Exo-glucanases adsorb onto cellulose microfibrils at the sites where free chain ends are available and proceed along the chain releasing cellobiose. Some enzymes are bound unproductively. Exo-glucanases mostly exist in two forms, CBHI and CBHII. CBH I proceeds from reducing end of the chain and CBH II from the non-reducing end. [96] The process of glucose release from cellulose is depicted in Figure 3.

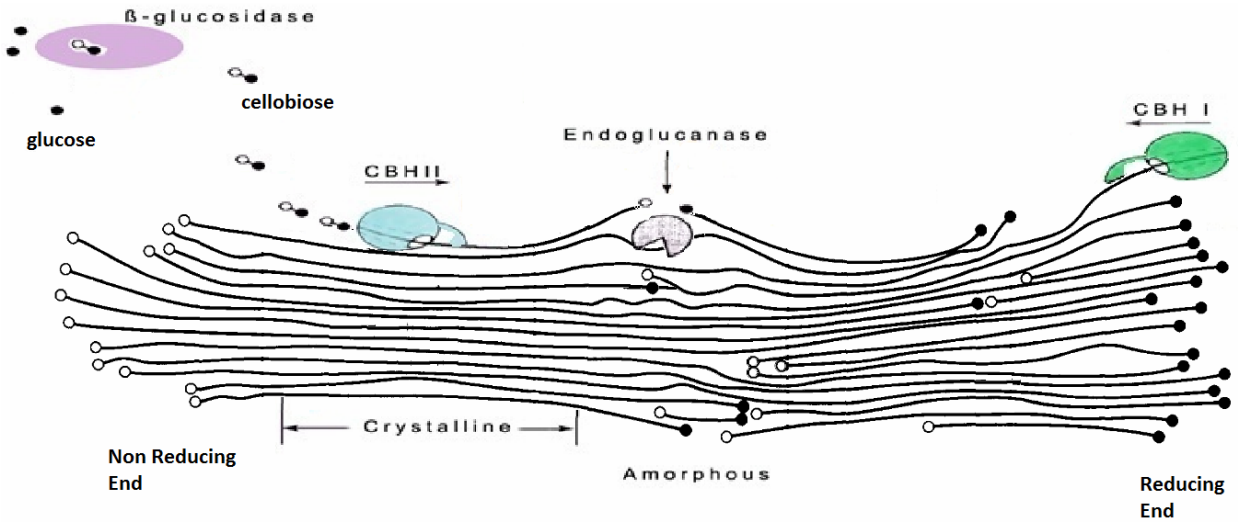


Figure 3. Depiction of enzymatic hydrolysis of cellulose by respective enzymes (modified from [96])

Enzymatic saccharification of cellulose takes place at optimum temperature of 45-50ºC and pH of 5.0. Cellulase loading range of 10-15 FPU/g dry substrate and b-glucosidase loading of 10-15 IU/g substrate are commonly used [62, 65, 51], although excessive cellulase loading (up to 50 FPU/g) is sometimes used to added to determine the effect of pretreatment on substrate digestibility[36]. Solids content is usually below 10% and enzymatic hydrolysis of most pretreated substrates is finished after 72h [62, 65, 66, 36, 68, 79, 73, 85, 51, 87]. Pretreatment conditions for different technologies that result in maximum enzymatic hydrolysis yields are shown in Table 5.

Cellulolytic enzymes are primarily inhibited by end products. Cellobiose is a more potent inhibitor of endo- and exo-glucanases than glucose which inhibits primarily β-glucosidases. The drop in the rate of cellulose hydrolysis is thus attributed to cellobiose accumulation [98, 99]. Commercial cellulase preparations do not contain enough B-glucosidase activity and must be supplemented with β-glucosidase preparation for efficient and extensive hydrolysis of cellulose [100]. Various other compounds and solvents can inhibit the action of cellulases. Ethanol and butanol were found inhibit the enzymes by denaturation, while acetone was found to be an activator of up to concentration of 79g/L beyond which inhibitory effect was also observed [101].

Depending on the mode of action, hemicellulases are divided into glycoside hydrolases or carbohydrate esterases. Similar to cellulases, xylanases hydrolyze β-1,4 bond in the xylan backbone, releasing short xylooligomers that are hydrolyzed into xylose by β-xylosidases [102]. The presence of xylan substituents contributes to resistance of xylan to xylan-degrading enzymes (Figure 4). And such a whole suite of accessory enzymes is required for efficient xylan hydrolysis such as α-arabinofuranosidases and α-L-arabinases that release arabinan [31], α-glucuronidases that release glucoronic acids, acetyl xylan esterases that hydrolyze acetylester bonds, ferulic acid esterases that hydrolyze p-coumaryl ester bonds and p-coumaric acid esterases that hydrolyze p-coumaryl ester bonds [37, 102].

Hemicellulose acts as a physical barrier to the action of cellulases and supplementing xylanase preparations can enhance enzymatic hydrolysis of both cellulose and hemicellulose [100, 26]. The effect holds true for all pretreatments with the benefit of xylanase supplementation depending on the type of pretreatment with total sugar yield boost between 40-100% [26]. An integrated study of effect of xylanase, as well as accessory enzymes on sugar yield of wheat straw pretreated by different technologies has not been reported. Such data, however, could be of great industrial importance.

Francisco et al. found that yield glucose increased from 14.6 g/l to 19.8 g/L and the xylose from 4.5 g/l to 5.5 g/l of steam-exploded wheat straw (210°C, 20 bar, 20 mm particle size and 10 min of reaction time) was supplemented with xylanase preparation during enzymatic hydrolysis [103].Pectin has similar steric effect on enzymatic hydrolysis of cellulose as hemicellulose [100]. Early study has shown that supplementing cellulase with pectinase increased sugar yield during enzymatic hydrolysis of wheat straw indicating that this structural component should be given consideration during bioprocessing of wheat straw [25].

Table 5. Optimum pretreatment conditions for maximum sugar yield during subsequent enzymatic hydrolysis of wheat straw pretreated by different technologies

Pretreatment	Screen size	Reaction Conditions	Sugar Yield after EH	Ref.
Steam	_____	180°C, 10 min, 0.9% w/w H_2SO_4,	85% (glucose)	[62]
Liquid Hot Water	5mm	214°C, 2.7min	90.6% (glucose)	[65]
Dilute Acid	1.27 mm	121°C, 1h, 0.75% H_2SO_4	74% (overall)	[66]
	1mm	170°C, 50 min, 89 mM maleic acid	100% (glucose) 90% xylan	[36]
Lime	1.27mm	121°C, 1hr, 100 mg $Ca(OH)_2$/g biomass	82% (overall)	[68]
Alkaline H_2O_2	1.27mm	35 °C, 24 h, pH 11.5, 2.15% H_2O_2 v/v	96.7% (overall)	[79]
Ammonia Percolation	1mm	170°C, 30min, 15% v/v NH_3	95% (glucose)	[73]
Organosolv	20mm	220°C, 3h, 20g aqueous glycerol(70%)/g biomass	90% (glucose)	[85]
Ozonolysis	1mm	4°C, 2.5h, 3% w/w O_3	84% (glucose)	[51]
Ionic Liquid	0.5cm	120°C, 30min, [EMIM]DEP	54.8% (glucose)	[87]

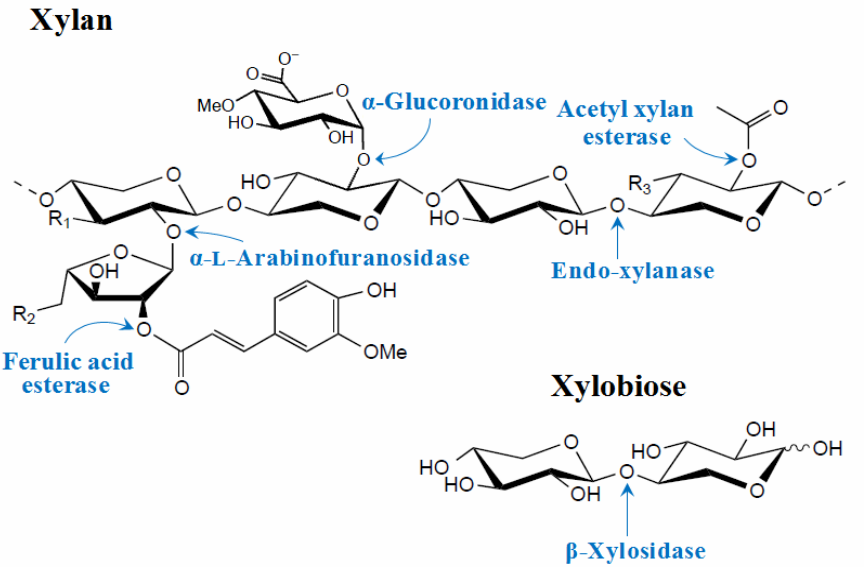


Figure 4. Xylan-degrading and accessory hemicellulases (modified from reference [95])

13. Additives

Utilization of various additives such as surfactants (i.e. Tween), non-catalytic proteins such as BSA or polyethylene glycol (PEG) to aid in enzymatic hydrolysis is has gained momentum in recent publications [104,105]. The effect of additives on enzymatic digestibility of wheat straw pretreated in pilot scale by different pretreatment methods is reported in [104]. Acid pretreated substrate gained the most benefit

from additives, for which the increase in cellulose conversion ranged from 58% (BSA) to 70% (Berol 08). For other pretreatments, cellulose saccharification extended by 3-23%. Improvements in xylan conversion were in the order of 0-10%, except for 11-17% for steam exploded straw. The optimum surfactant loading was found to be 0.05 g/g dry biomass. The major effect of surfactants is prevention of unspecific binding of enzyme to lignin while BSA acts by binding to lignin instead of the enzyme [104]. These additives could benefit biotechnological processes by increasing yields and reducing the amount of enzyme required. Improvements to enzyme recycling by surfactants are also reported [106].
Functional proteins such as expansins and swollenins can also aid in enzymatic hydrolysis of lignocellulose. Expansins facilitate enzymes by disrupting hydrogen bonding in the packaging of the plant cell wall and polysaccharides [107, 108]. Synergism, as high as 240%, was found between bacterially produced expansin and cellulase during enzymatic hydrolysis of filter paper at low dosage of enzyme [108]. More research is required to estimate the feasibility of using these compounds for enzymatic hydrolysis of lignocellulose.

14. Strategies to reduce enzyme cost

Continuous reductions in enzyme cost are offered by alternate process design and genetic engineering. Strategies identified to reduce enzyme cost include increasing enzyme production efficiency, increasing enzyme specific activity and recycling of enzymes to be used in subsequent hydrolysis [106].

14.1 Enzyme production

A stirred tank reactor is widely used for the production of lignocellulolytic enzymes, however it is known to have shear problems that lead to rupture of mycelia cells and deactivation of enzymes. Alternatively air-lift or bubble column bioreactors alleviate such shear stress problems resulting in better yield and productivity[9]. On site enzyme production has shown to have unique advantages for the ethanol plant site: eliminating the need for stabilizers against microbial contamination and protein denaturation used during enzyme storage and utilizing hydrolysis sugar as a cost–effective feedstock for enzyme production [8]. Fungal co-culturing of T. *reesei,* that exhibits high CBH and EG activities while lacking sufficient B-glucosidase activity, with A. *niger*, that secrets excess of B-glucosidase while lacking in EG activity, was shown to result in enzyme cocktails with enhanced cellulolytic activity (synergy for hydrolysis of cellulose) [109]. Enzymes can also be produced on pretreated substrate which acts as an inducer resulting in increased activity of secreted enzyme mixtures [109, 110].

14.2 Progress in improving enzyme specific activity

Cloning of thermostable cellulolytic enzymes from thermophillic bacteria into *Trichoderma reesei* resulted in shift in operating temperature of the enzymes to the range 55-60ºC as compared to the operating temperature of 45-50ºC of conventional enzymes. As a result, the protein dosage of thermophillic enzymes required to achieve the same conversion as at 45-40 C is decreased as the specific activity of the enzymes at 55-60ºC is increased. Synergy and effectiveness of thermostable enzyme mixtures can be further improved by developing cloning techniques for a wider range of cellulases and accessory enzymes[111]. Cloning of high glucose tolerant β-glucosidases can also result in reductions of protein required for the process.
Mutagenesis of enzyme producing fungal strains by chemicals, such as N-methyl-N'-nitro-N-nitrosoguanidine (NTG), and UV-light is widely used to screen for hyper-producing strains. T. reesei mutants producing 4-5 times more cellulase than the wild type strain and *Penicillium verruculosum* mutants secreting 3 times more cellulase and xylanase were developed using these techniques. Site-directed mutagenesis methods such as saturation mutagenesis, error-prone PCR and DNA shuffling have been used to improve specific enzyme properties such as thermostability, catalytic performance and stability at high pH (i.e. 6.2) [109].

14.3 Enzyme recycling

There are many strategies to recycle enzymes and reduce the amount of enzymes required for the process (Figure 5). The enzymes adsorbed on residual substrate after sacharification can be recycled by suspending residual substrate together with a fresh substrate, or a fresh substrate can be continuously added to the hydrolysis reactor by a fed-batch principle. Temperature, pH and surfactant concentration were determined to be the major factors affecting enzyme desorption from residue substrate. Increase in enzymatic hydrolysis by 25% could be achieved after three rounds of recycling [106].

Ultra filtration (UF) membranes of narrow molecular weight cutoff (50kDa) can sieve the sugars through while retaining the enzymes. Virtually eliminated end product inhibition has shown to increase the hydrolysis rate and reducing sugar yield of steam-exploded corn stalk by 200% and 206% as compared to batch operation. Dilute sugar stream in permeate can be found disadvantageous on the other hand [112]. Permeate flow rate is crucial parameter for successful operation of a membrane reactor. When the flow rate exceeds a critical point, deactivation of enzyme due to migration to concentration polarization layer where the shear from stirring is high may become significant [113]. If 75% of the enzyme is recycled in active form, the cost benefit of membrane filtration may be as much as 18 cents/gal of ethanol produced [114].

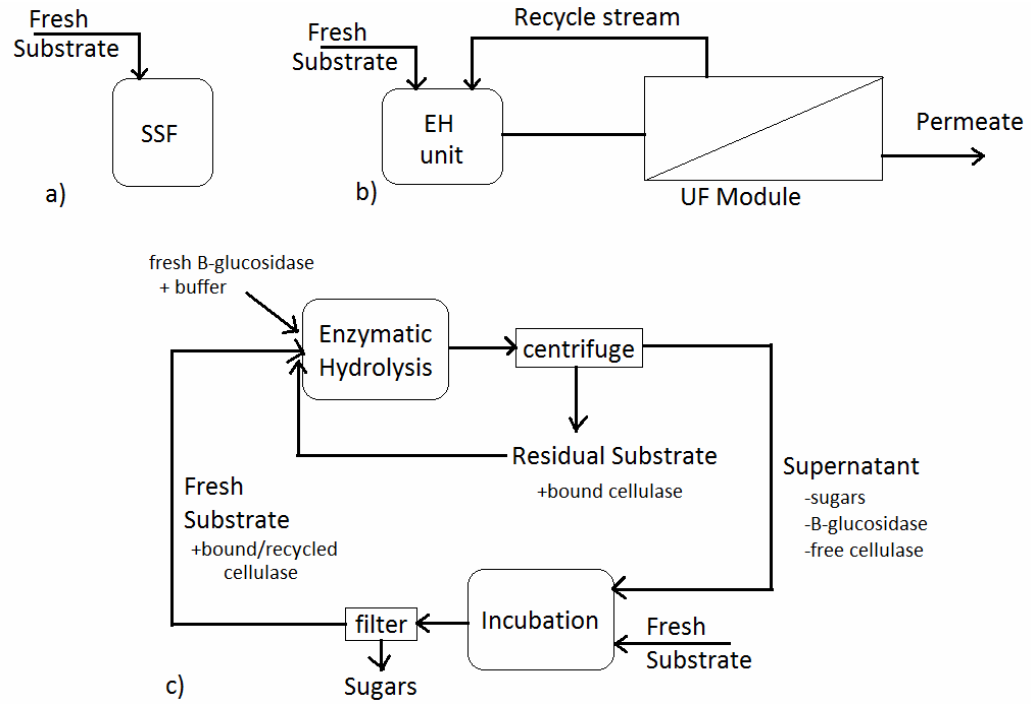


Figure 5. Plausible ways of recycling enzymes: (a) fed-batch simultaneous saccharification and fermentation (SSF); (b) continuous ultra filtration (UF) and (c) recycling of free cellulose from hydrolysis supernatant

15. Enzymatic hydrolysis at high solids content

Enzymatic hydrolysis at higher solids loading is desirable from an industrial point of view. Solid content of 10-15% is required to achieve ethanol yields of 4-5%. Increasing the solid loading above that would be desirable as it would result in higher ethanol yields, however end product inhibition would limit sugar yield in such case. To eliminate end product inhibition enzymatic hydrolysis and fermentation processes are combined together in what is called simultaneous saccharification and fermentation (SSF), where fermenting microorganisms continuously consume released sugars. Although optimum temperature for enzymatic hydrolysis is 45-50ºC while it is 30ºC for fermentation, the process is still able to achieve conversion. In conventional stirred tank reactors operating at solid content above 10-15% becomes impossible due to high viscosity. This objective can be achieved by SSF and the pretreated solids are usually liquefied prior to SSF. At IBUS, a special liquefaction reactor consisting of paddled horizontally placed gravimetric mixing drum can handle solid concentrations up to 40% (w/w). The pretreated straw can be completely liquefied within 4 hours. It was found however that enzymatic conversion of both hemicellulose and cellulose were linearly decreasing when solids content increased from 2% to 40%. Cellulose conversion gradually decreased from 70% at 20% solids to 49% at 40% solids [115]. The decrease in enzymatic hydrolysis yield is not yet fully understood. It was found that while neither lignin content nor hemicellulose derived inhibitors were responsible for a decrease in yields, product inhibition could not account the decrease in conversion. Enzyme adsorption studies showed that inhibition of cellulase adsorption was responsible for a decrease in yield and it was hypothesized that inhibition of cellulose binding domain by high glucose and cellobiose concentrations was behind it [116]. Additional

research is required to gain better understanding of the process as overcoming this bottleneck could result in a breakthrough in ethanol yields.

16. Future perspective for enzymatic hydrolysis of lignocellulose

Enzymatic hydrolysis of lignocellulose is a complex event that requires finely tuned synergy between number of enzymes to achieve maximum sugar yield. To date most research has been focused on improving the performance cellulolytic enzymes for commercial processes. However, with fermenting strains for both butanol and ethanol capable of utilizing both glucose and xylose, enzymatic hydrolysis of hemicellulose needs to get equal attention. Both xylanase supplementation and use of surfactants could benefit commercial lignocellulose based bioprocesses as they have shown great improvements in sugar yields for steam explosion pretreated wheat straw.

Leading enzyme research companies, Novozyme and Genencor, have recently announced a breakthough in creating commercially viable enzyme mixtures. Novozyme's new Cellic CTec2 enables the bioethanol industry to produce bioethanol at $2.00 per gallon which matches current market prices. The enzyme works with a range of lignocellulosic substrates and brings the enzyme cost down to $0.50 per gallon of cellulosic ethanol. Genencor's Accellerase DUET achieves higher sugar yields at 3 times lower dosing essentially reducing enzyme cost for bioprocesses. The enzyme costs have dropped 80% over the past 2 years [117]. With a trend of constant improvement, further research could still reduce enzyme cost.

17. Conclusion

Crop residue wheat straw is a highly abundant lignocellulosic feedstock throughout the world offering its rich sugar platform for large scale development by the biofuel industry. Most pretreatment methods in the literature were shown to be successful at overcoming recalcitrance of wheat straw making it highly amenable to the action of hydrolytic enzymes. Steam explosion pretreatment and liquefaction of lignocelluloses at high solids content has shown great potential for large scale commercialization, although further research is required to gain better understanding of impediments to the action of enzymes at high biomass consistency. Enzymatic hydrolysis has great potential for improvement in both enzyme production, recycling and genetic screening areas. As opposed to current biomass utilization approach, where lignin is used to power the energy requisites of the process, development of lignin platform for production of biochemicals and products as well as extraction of value added chemicals from wheat straw such as wax, pectin, phenolic acids can set for a new stage in the biorefining industry.

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